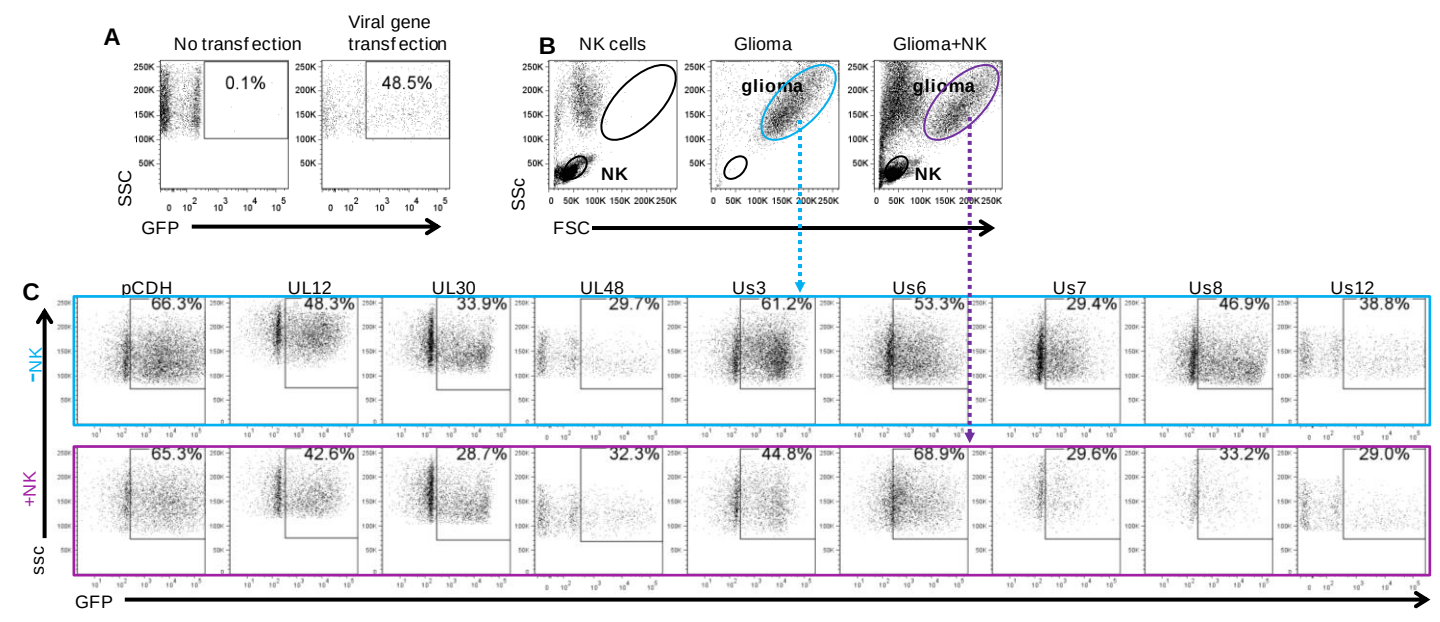
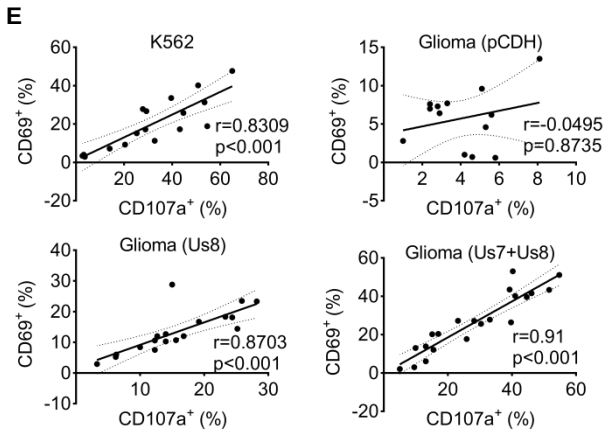
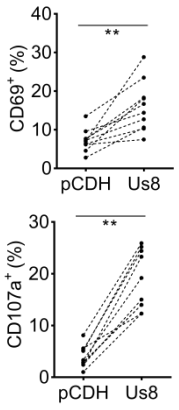


D

gene	pCDH	UL12	UL30	UL48	Us3	Us6	Us7	Us8	Us12
$\Delta GFP = \frac{(+nk)\% - (-nk)\%}{(-nk)\%}$	$\frac{65.3-66.3}{66.3}$	$\frac{42.6-48.3}{48.3}$	$\frac{28.7-33.9}{33.9}$	$\frac{32.3-29.7}{29.7}$	$\frac{44.8-61.2}{61.2}$	$\frac{68.9-53.3}{53.3}$	$\frac{29.6-29.4}{29.4}$	$\frac{33.2-46.9}{46.9}$	$\frac{29.0-38.8}{29.0}$
Relative Cytotoxicity	-1.50%	-11.80%	-15.34%	8.75%	-26.80%	29.27%	0.68%	-29.21%	-25.26%
Effect (cut-off value ±7.5%)	null	susceptible	susceptible	resistant	susceptible	resistant	null	susceptible	susceptible





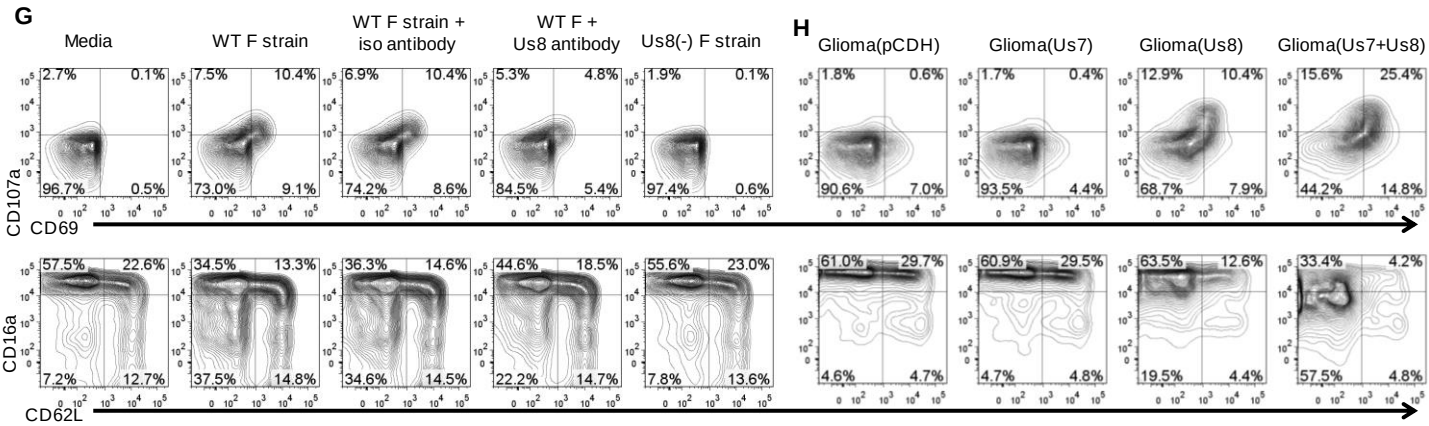


Figure S1. HSV1 gE activated NK cells, related to Figure 1, 2. (**A**) GFP reports the expression of HSV1 viral genes. B-D, Gating strategy (**B**), representative graphs (**C**), and sample result analysis (**D**) for DC-MEGE assay. Human primary NK cells and living glioma cells show distinctive size (measured by FSC) and internal complexity (measured by SCC), and thus are separated easily without labelling(**B**). pCDH is the empty vector for cloning and expressing individual HSV1 genes. Transfection efficacy was not 100%, so both GFP+ cells and GFP- cells exist after transfection. 24 hours after transfection, glioma cells were cultured with ((+)NK) or without ((-)NK) primary human NK cells for 5 hours, and living glioma cells were gated as in (B) and percentage of GFP+ glioma cells was recorded (**D**). (**D**) Quantitation of the DC-MEGE assay for representative HSV1 genes show in c. Here shows only the results from one human donor. Complete DC-MEGE results of all HSV1 genes were shown in **Figure 1B**. (**E,F**) Phenotypes of primary human NK cells after 7-hour culture in media, with K562 cells (positive control), or transfected glioma cells. (**E**) Percentage of NK cells positive for CD69 were plotted against the percentage of NK cells positive for CD107a in corresponding human donors, each represented by one dot. Correlation of CD69 and CD107a were calculated using linear regression (n=13-17). (**F**) Percentages of NK cells gaining the expression of CD69 or CD107a in random normal donors. Data summarized for 10 donors. Each dotted line in (**F**) links data acquired from the same donor. ** $p<0.01$. (**G**, Representative phenotype of primary human NK cells after 7 hours of culture in plates coated with inactivated pure WT HSV1 F strain or Us8 deficient HSV1 F strain viruses. Isotype or anti-Us8-specific antibody was added to interfere with the interaction between NK cells and coated F strain viruses. Representative contour staining of 7 donors is shown. (**H**) Phenotype of primary human NK cells after 7 hours culture with glioma cells transfected with pCDH or different HSV1 genes. Representative contour plot from one of 8 donors is shown, and statistical summary is shown in Figure 2A.

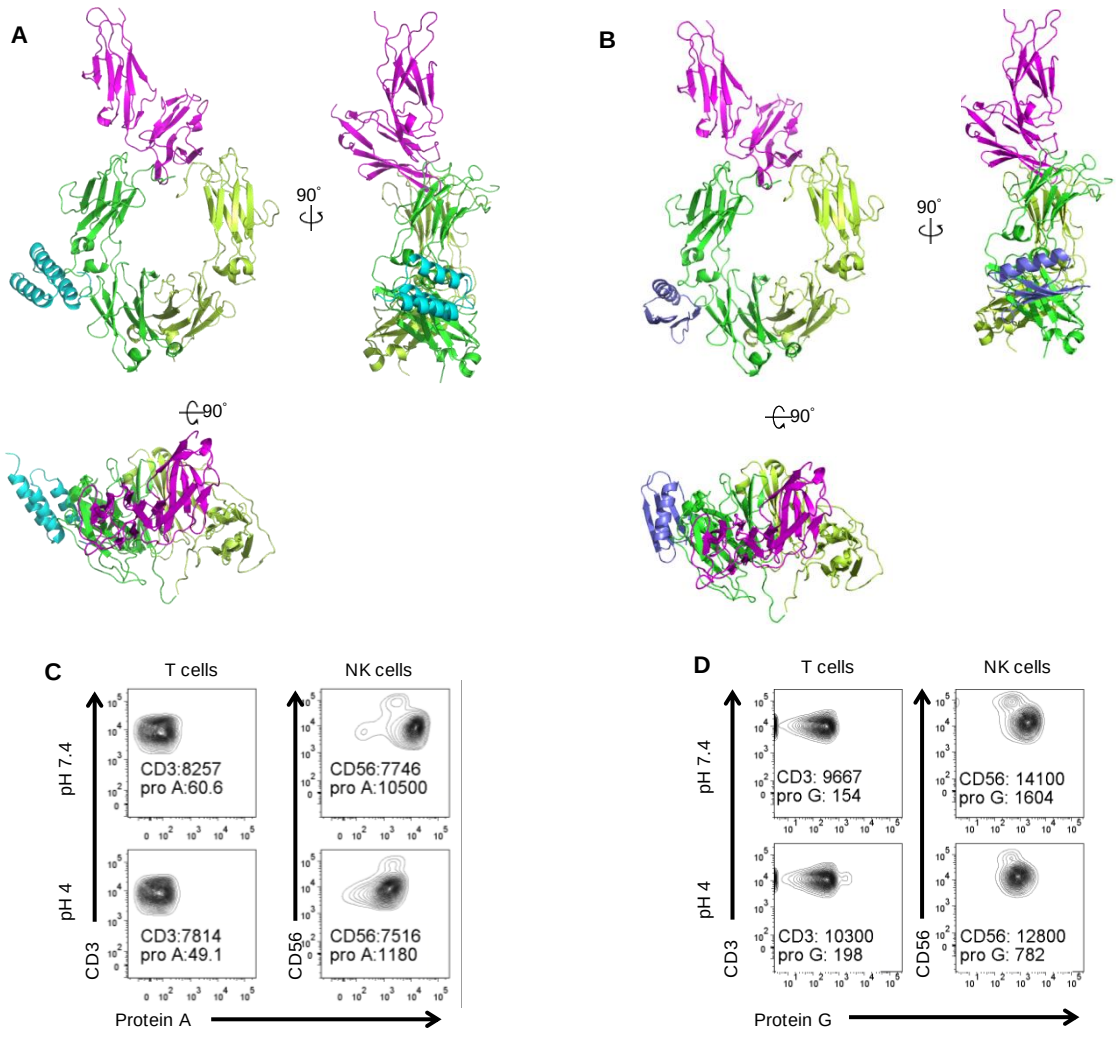


Figure S2. IgG Fc bridges CD16a and IgG binding proteins from pathogens, related to Figure 3. (A, B) Model structure of protein A-IgG1Fc-CD16a (**A**) or protein G-IgG1Fc-CD16a (**B**) ternary complex. Front view, side view and top view are shown respectively. CD16a is shown as magenta, two monomers of IgGFc dimer are shown as green and lime, protein A is shown as cyan, protein G is shown as purple blue. (**C, D**) Binding of protein A (**C**) or protein G (**D**) to human PBMCs, which were briefly washed with pH7.4 or pH4 media prior to staining. Numbers in e, f are mean intensity of each staining. Data in (**C**) and (**D**) are the representative of at least 4 repetitions.

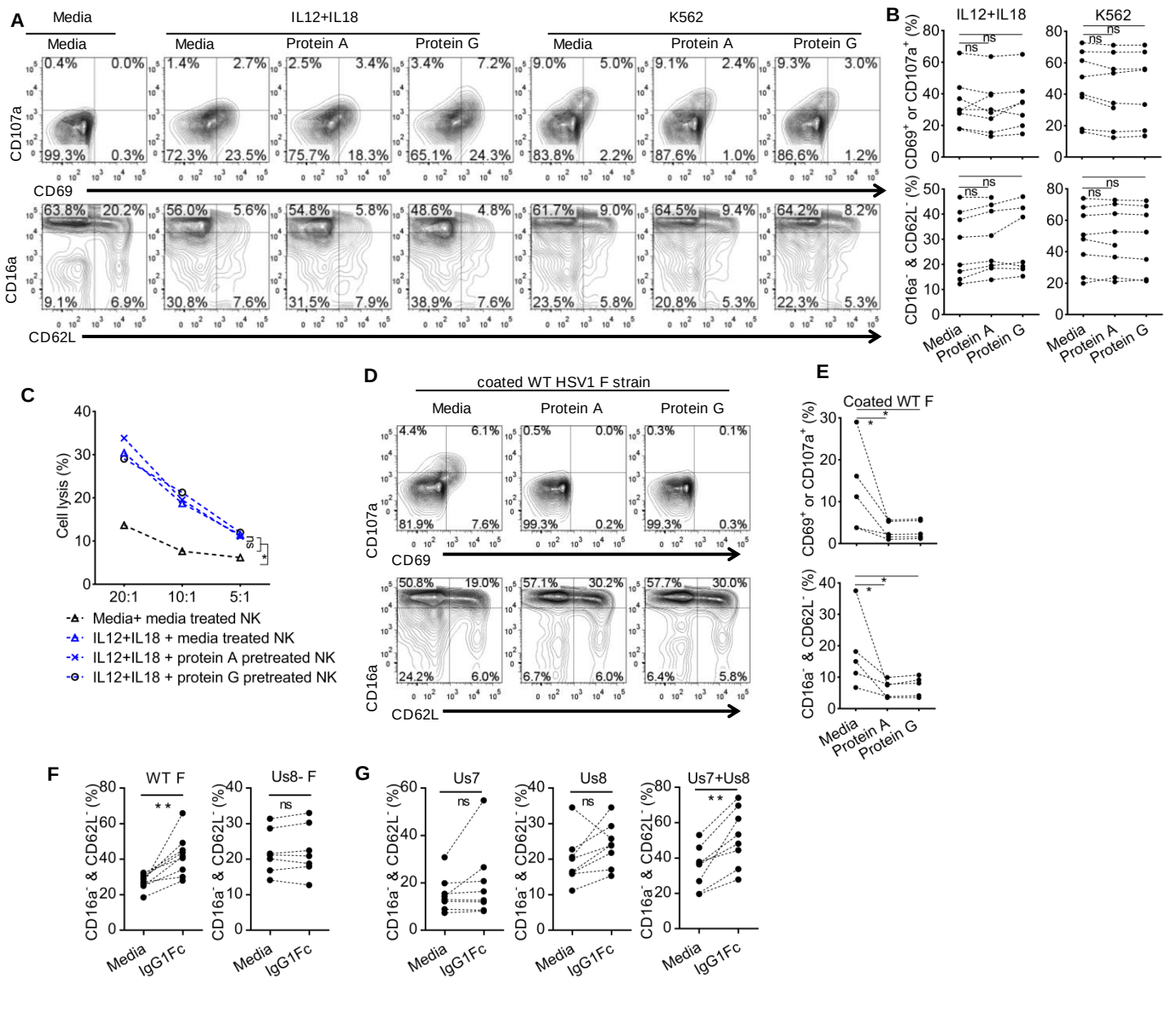


Figure S3. IgG binding is essential for HSV1 gE to activate human NK cells, related to Figure 4. (A, B) representative phenotypes (**A**) and statistical summary (**B**) of primary human NK cells, which were first incubated with media, or media supplemented with protein A or protein G for 30 minutes, followed by culturing with different stimuli for another 7 hours (n=7-8). (**C**) Cytotoxicity of primary human NK cells, which were pretreated with media, protein A or protein G for 30 minutes, against glioma cells in the absence or presence of IL12+IL18 (data are mean of triplicates, and one representative of three experiments is shown). (**D,E**) Human NK cells were treated with medium alone, protein A or protein G, and subsequently cultured in plates coated with inactivated WT HSV1 F strain. Phenotyping was performed after 7 hours of culture. **(F, G)** NK cells were cultured with glioma cells infected with WT or Us8- HSV1 viruses (**F**), or glioma cells transfected with HSV1 genes (**G**), in the absence or presence of human IgG1Fc, and stained after 7 hours of culture. Statistical summary for the loss of CD16a and CD62L (n=7-9) donors were shown, statistical summary for the increase of CD69 and CD107a were shown in Figure 4D and E. * p<0.05.** p<0.01.

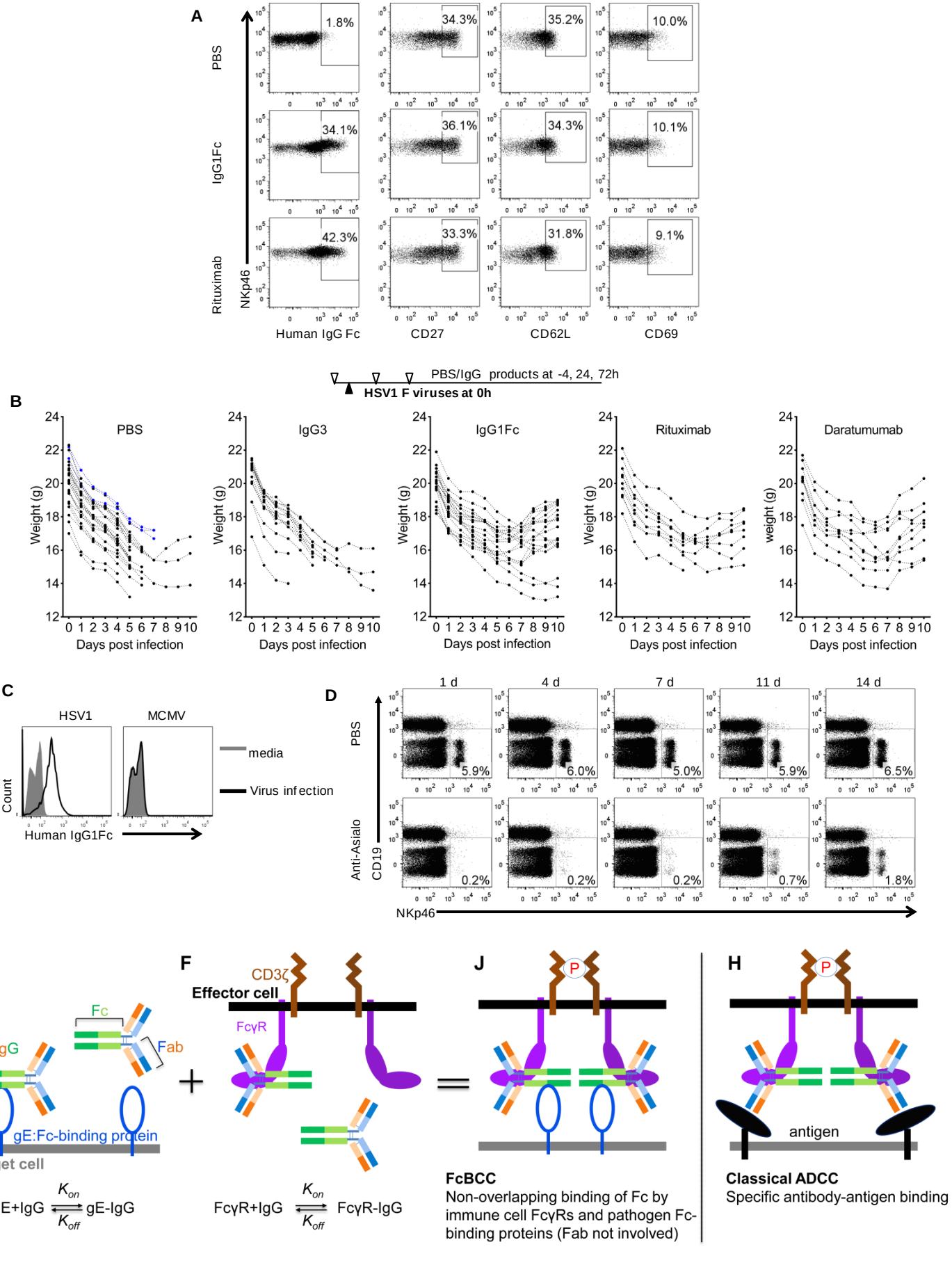

A
PBS
IgG1Fc
Rituximab
NKp46
Human IgG Fc
CD27
CD62L
CD69
1.8%
34.3%
35.2%
10.0%
34.1%
36.1%
34.3%
10.1%
42.3%
33.3%
31.8%
9.1%
PBS/IgG products at -4, 24, 72h
HSV1 F viruses at 0h
B
PBS
IgG3
IgG1Fc
Rituximab
Daratumumab
Weight (g)
weight (g)
Days post infection
C
HSV1
MCMV
Count
Human IgG1Fc
media
Virus infection
D
1 d
4 d
7 d
11 d
14 d
PBS
Anti-Asialo
CD19
NKp46
5.9%
6.0%
5.0%
5.9%
6.5%
0.2%
0.2%
0.2%
0.7%
1.8%
E
Fc
IgG
Fab
gE:Fc-binding protein
Target cell
gE+IgG
gE-IgG
F
CD3ζ
Effector cell
FcγR
FcγR+IgG
FcγR-IgG
J
P
FcBCC
Non-overlapping binding of Fc by immune cell FcγRs and pathogen Fc-binding proteins (Fab not involved)
H
P
antigen
Classical ADCC
Specific antibody-antigen binding

Figure S4. **FcBCC protect mice from lethal HSV1 infection, related to Figure 5.** (**A**) Balb/c mice were i.p. injected with PBS, human IgG1Fc or rituximab, and bleed 24 hours later. PBMC were stained with lineage markers, mouse anti human Fc antibody, and other mouse NK cell activation markers. One representative of three mice was shown. (**B**) Weight change of mice after receiving the injection regimen noted above. Mice were weighted before (0 day) and at every 24 hours after virus challenge till death or the tenth day of infection. Data linked by dotted line are from the same mouse. Two mice in PBS group (blue dots and lines) were alive till the end of the survival study but weight data were missed since 8^{th} day. Missing of these data did not affect the general conclusion of this study. (**C**) BHK cells were infected with WT HSV1 or MCMV at MOI=0.1 for 24h and tested for the binding of human IgG1Fc to virus-infected cells. (**D**) Depletion of NK cells by anti-Asialo GM1 antibody. Mice were i.p. injected with 20ug anti-Asialo GM1 antibody or equivalent volume of PBS, and bleed at specific time points. Blood monocytes were stained with lineage markers. NK cells are gated CD19-NKp46+ cells. One representative of three experiments was shown. (**E-H**) Schematic model and comparison of NK cell activation by the FcBCC and the classical ADCC.

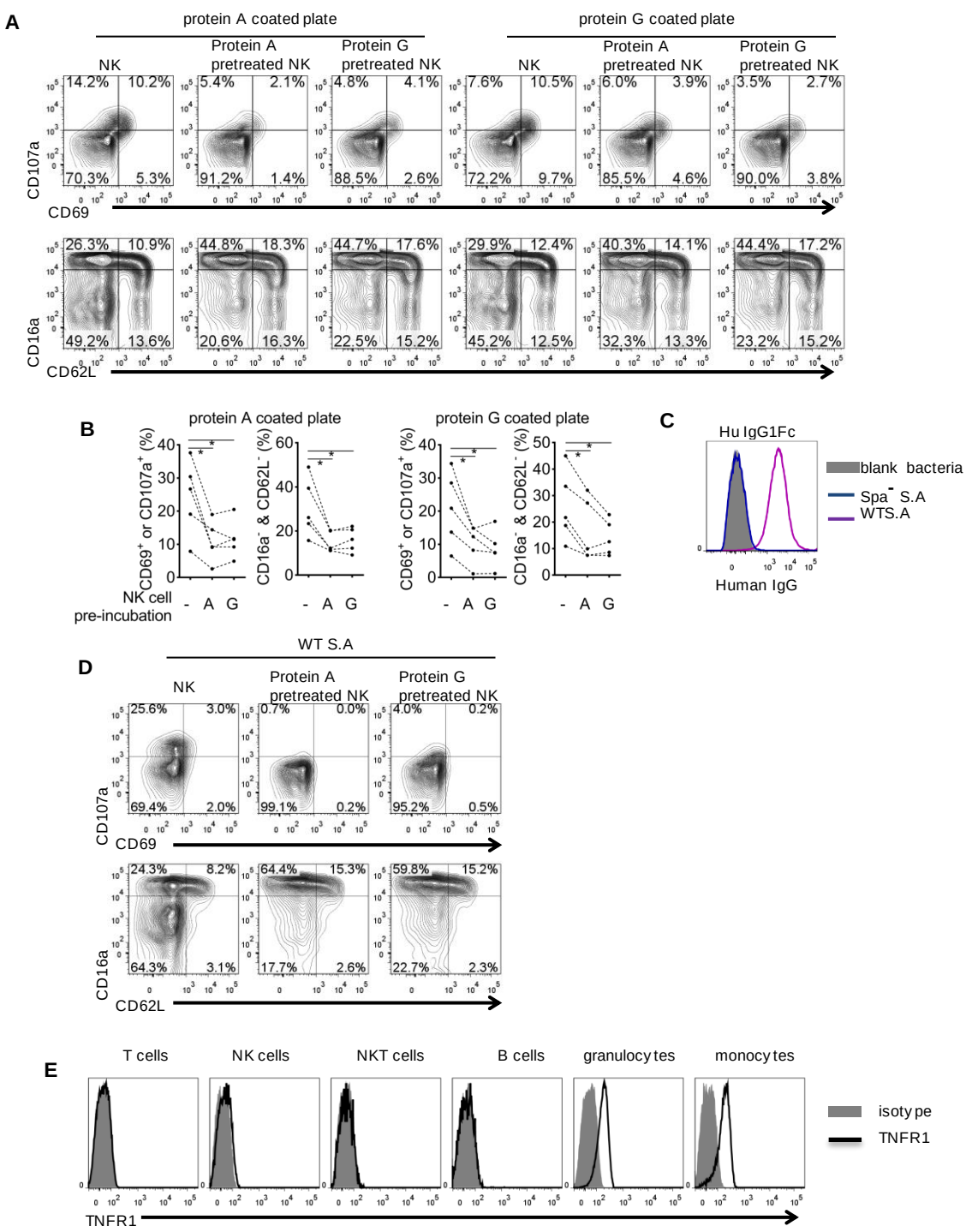


Figure S5. **Polymerized protein A and protein G activate NK cell through IgG bridge ,related to Figure 6.** (**A, B**) representative phenotype (**A**) and statistical summary (B) of primary human NK cells that were incubated with soluble protein A or protein G for 30min, and subsequently cultured in protein A or G-coated plates for 7 hours (n=5). (**C**) S.A bacteria were incubated with fluorescent human IgG and analyzed with flow cytometry to test the presence of IgG binding proteins. One representative of three experiments is shown. (**D**) Phenotype of NK cells that were pretreated with soluble protein A or protein G, and subsequently cultured with WT S.A for 7 hours. One representative of four donors is shown. (**E**) Expression of TNFR1 in different subsets of human PBMC. * $p<0.05$