Supporting Information

Systemic delivery of glycosylated-PEG-masked oncolytic virus enhances targeting of antitumor immuno-virotherapy and modulates T and NK cell infiltration

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**Figure S1.** The gate and EGFP intensity of oHSV-treated Hepa-1-6 cells were analyzed by FACS.

**Figure S2.** The TEM image of oHSV and glycosylated-PEG-oHSV.
Figure S3. Standard curve for virus copy number determination.

Figure S4. Flow cytometry was performed on HepG2 cells treated with oHSV or glycosylated-PEG-oHSV. Statistical analysis was performed with t-test, *p<0.05, (n = 3). Data are presented as mean ± SD.
**Figure. S5.** The gate and EGFP intensity of NIH/3T3 cells treated with oHSV were analyzed by FACS.

**Figure. S6.** Flow cytometry analysis and quantification of EGFP expression in NIH/3T3 cells after treatment with oHSV or glycosylated-PEG-oHSV for 12 h. Statistical analysis was performed using a t-test analysis, and significance levels were denoted by *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. Data are presented as mean ± standard deviation (SD).
Figure. S7. The cytolytic activity of Hepa1-6 cells treated with oHSV for 24 h was analyzed by FACS using Annexin V-FITC and PI staining.
Figure. S8. Biochemical analysis, including albumin, ALB; alkaline phosphatase, ALP; alanine aminotransferase, ALT; aspartate aminotransferase, AST; the blood urea nitrogen to serum creatinine ratio (BUN/SCR); and total cholesterol (TCHO) was performed in mice at the day 24 after intravenous injection of PBS, oHSV, or glycosylated-PEG-oHSV (n=3). Statistical analysis was conducted using ANOVA analysis, with significance levels denoted as *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001. The data are presented as mean ± standard deviation (SD).
**Figure. S9.** Identifying the maturation of lymphatic dendritic cells (DCs) from lymph nodes of mice after receiving different treatments by FACS analysis with staining using anti-CD11c-APC, anti-CD80-PE, and anti-CD86-PE-Cy7 antibodies.

**Figure. S10.** Identifying the percentage of CD8⁺T cells in spleen after receiving different treatments by FACS analysis with staining using anti-CD3-APC, anti-CD8-PE antibodies.
**Figure S11.** The percentage of CD3+CD4+T cells in tumors after treatment with PBS, oHSV or glycosylated- PEG-oHSV, respectively (n = 5).

**Figure S12.** Identifying the percentage of CD8+ T cells in tumors after receiving different treatments by FACS analysis with staining using anti-CD3-APC, anti-CD8-PE antibodies.
Figure. S13. Identifying IFN-γ⁺CD8⁺CD3⁺T cells in tumors after receiving different treatments by FACS analysis with staining using anti-CD3-APC, anti-CD8-PE, IFN-γ-PE-Cy7 antibodies.

Figure. S14. Identifying NK cells in tumors after receiving different treatments by FACS analysis with staining using anti-CD3-FITC and anti-NK1.1-APC antibodies.
**Figure. S15.** Detection of Foxp3$^{+}$CD25$^{+}$CD4$^{+}$T cells in tumors following different treatments by FACS analysis with staining using anti-CD3-APC, anti-CD4-FITC, anti-CD25-PerCP-Cy5.5, and anti-Foxp3-PE-Cy7 antibodies.

**Figure. S16.** Hematoxylin and eosin (H&E) imaging was performed on major organs including the heart, liver, spleen, lung, and kidney, obtained from mice at the day 24 after intravenous injection of PBS, oHSV, or glycosylated-PEG-oHSV. Scale bar, 100 μm.