YMTHE, Volume *29*

Supplemental Information

Reshaping the Immune Microenvironment by Oncolytic Herpes Simplex Virus in Murine Pancreatic Ductal Adenocarcinoma

Liming Zhang, Wei Wang, Ruikun Wang, Nianchao Zhang, Hang Shang, Yang Bi, Da Chen, Cuizhu Zhang, Long Li, Jie Yin, Hongkai Zhang, and Youjia Cao

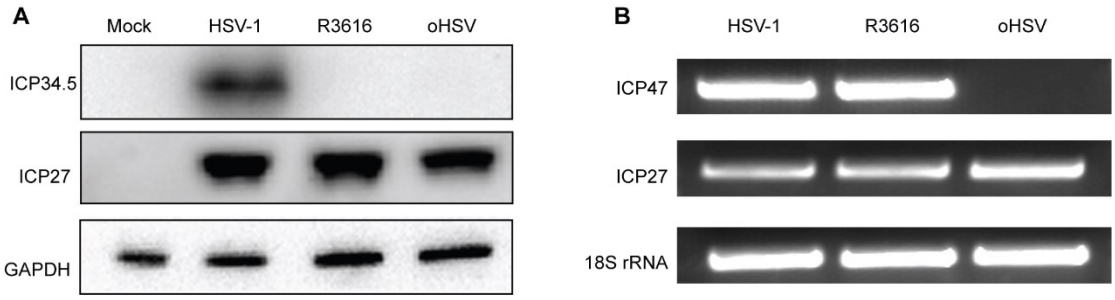

Figure S1. Verification of Engineered oHSV.

(A) HEK293T cells were mock infected or infected with HSV-1, R3616, oHSV at MOI = 1 for 48 hrs, respectively. Then cells were harvested and lysed for Western blot analysis with antibodies as indicated. (B) The infected HEK293T cells as above were collected at 48 hpi, then total RNA was extracted for RT-PCR analysis with primers for viral genes: *ICP47*, *ICP27* and cellular 18S rRNA respectively.

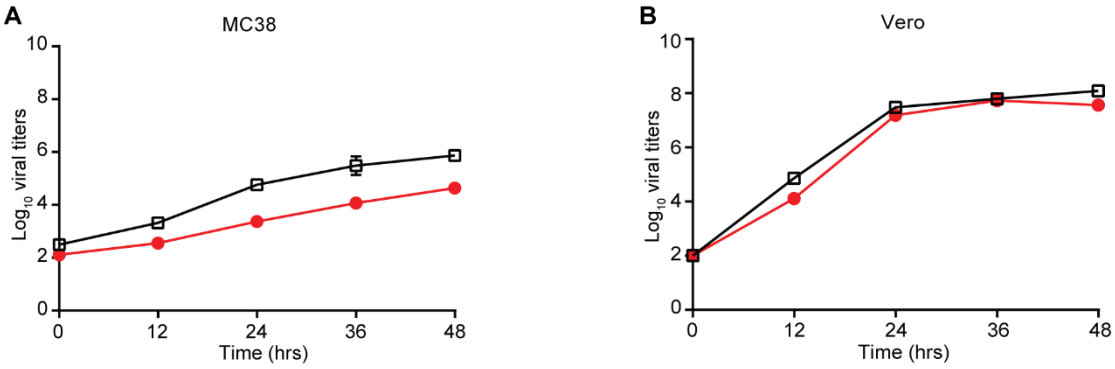

Figure S2. Titration Assay of oHSV Replication in Cell Lines.

(A) MC38, (B) Vero cells were infected with wild type HSV-1 (squares) or oHSV (circles) at MOI = 0.01, and the titers of viruses in these cells were determined at indicated time points as described in Materials and Methods. Viral replication was presented as Log of pfu per mL of the cultured medium and plotted on the vertical axis. Data represented the mean values from three independent experiments with the standard deviations as indicated.

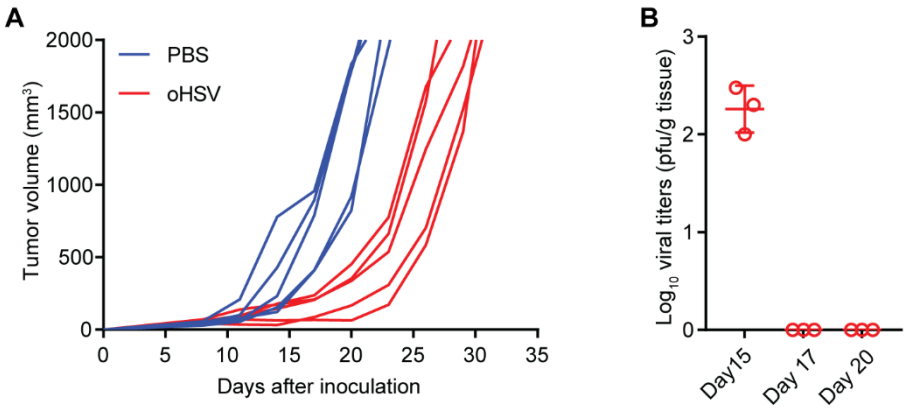

Figure S3. Titration of oHSV-Treated Tumor Samples.

(A) Tumor growth curve of individual mouse from PBS and oHSV treated group (n=5). (B) Tumor tissues were isolated from oHSV treated mice on days 15, 17, and 20, and the replication of oHSV was examined by viral titration assay as described in Materials and Methods. Data were presented as a Log of pfu per gram of tumor tissue and plotted on the vertical axis with standard deviations (n=3).

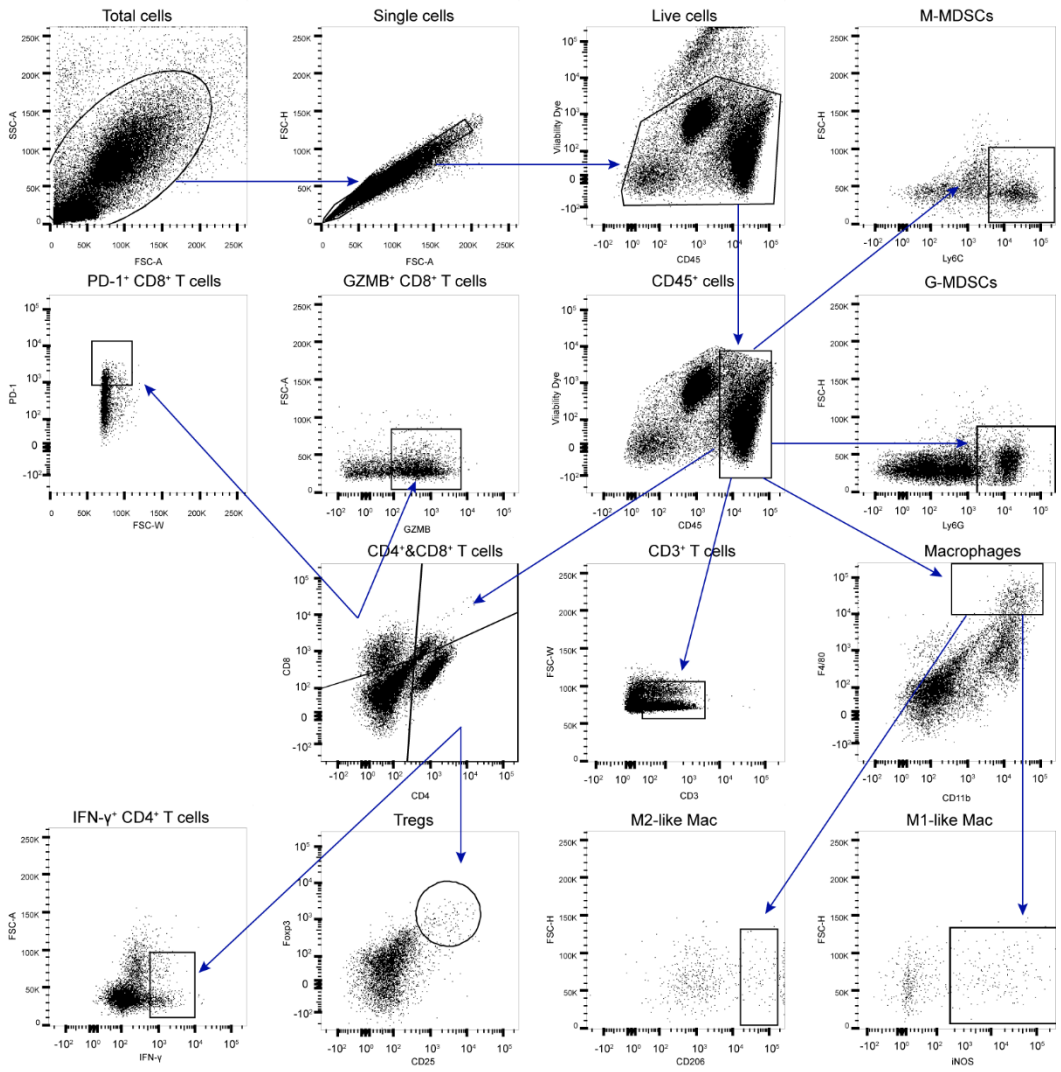

Figure S4. Gating Strategy of Flow Cytometry Analysis.

Gating strategy on tumor cell suspensions on indicated days to check immune cell infiltration. Samples were stained with indicated antibodies and subjected to flow cytometry analysis, and data were analyzed by FlowJo software.

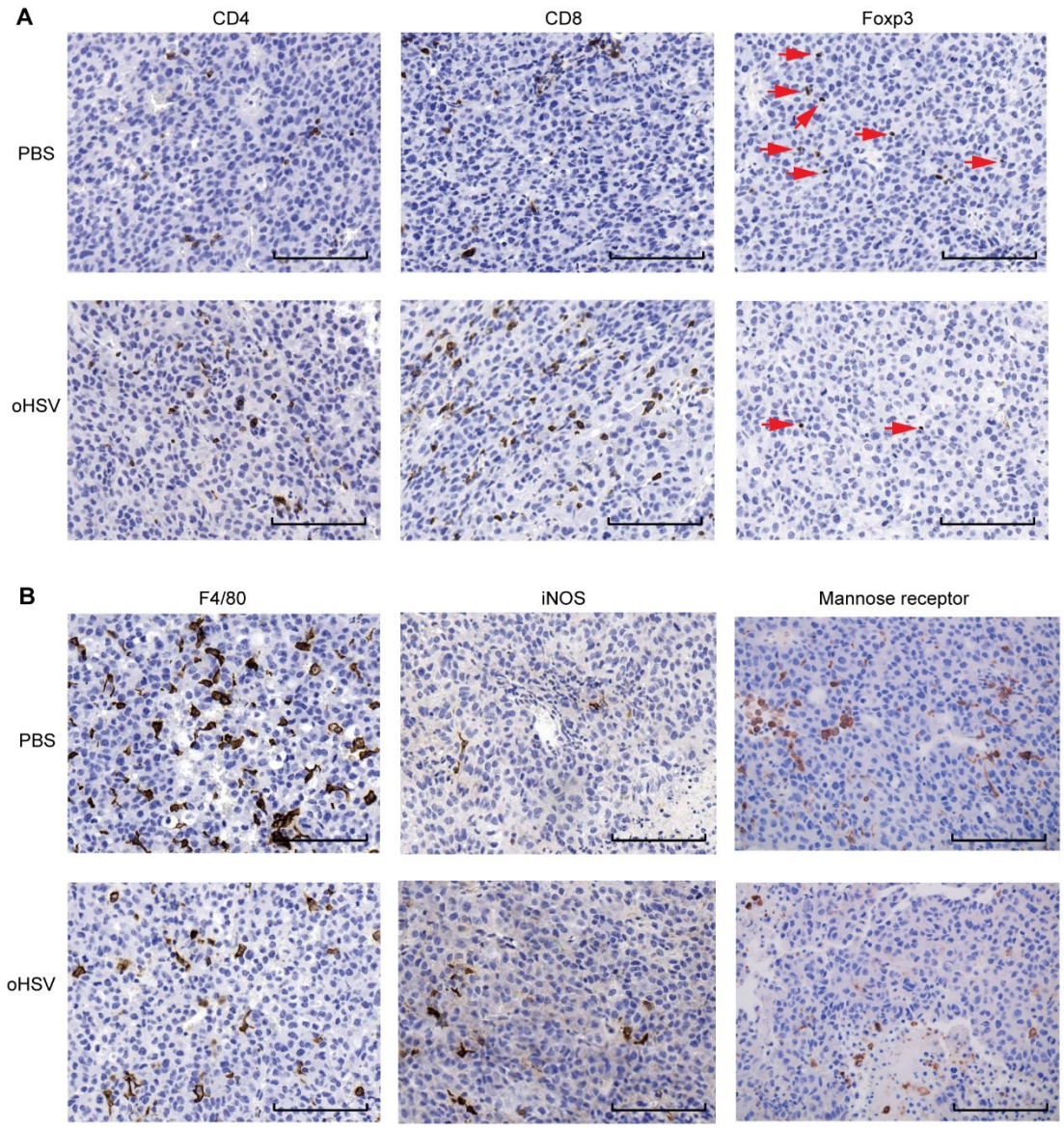

Figure S5. Immunohistochemical Staining of Tumor Infiltrating Cells in PDAC.

Tumor-bearing mice were treated with PBS or oHSV. On day 20, tumor tissues were collected and stained with T cell markers (A), anti-CD4, anti-CD8, and anti-Foxp3 antibodies; and macrophage markers (B), including anti-F4/80 (macrophages), anti-iNOS (M1-like macrophages) and anti-Mannose receptor (M2-like macrophages) antibodies. Representative images were presented. Tregs were highlighted with red arrows. Scale bars:100 μm.

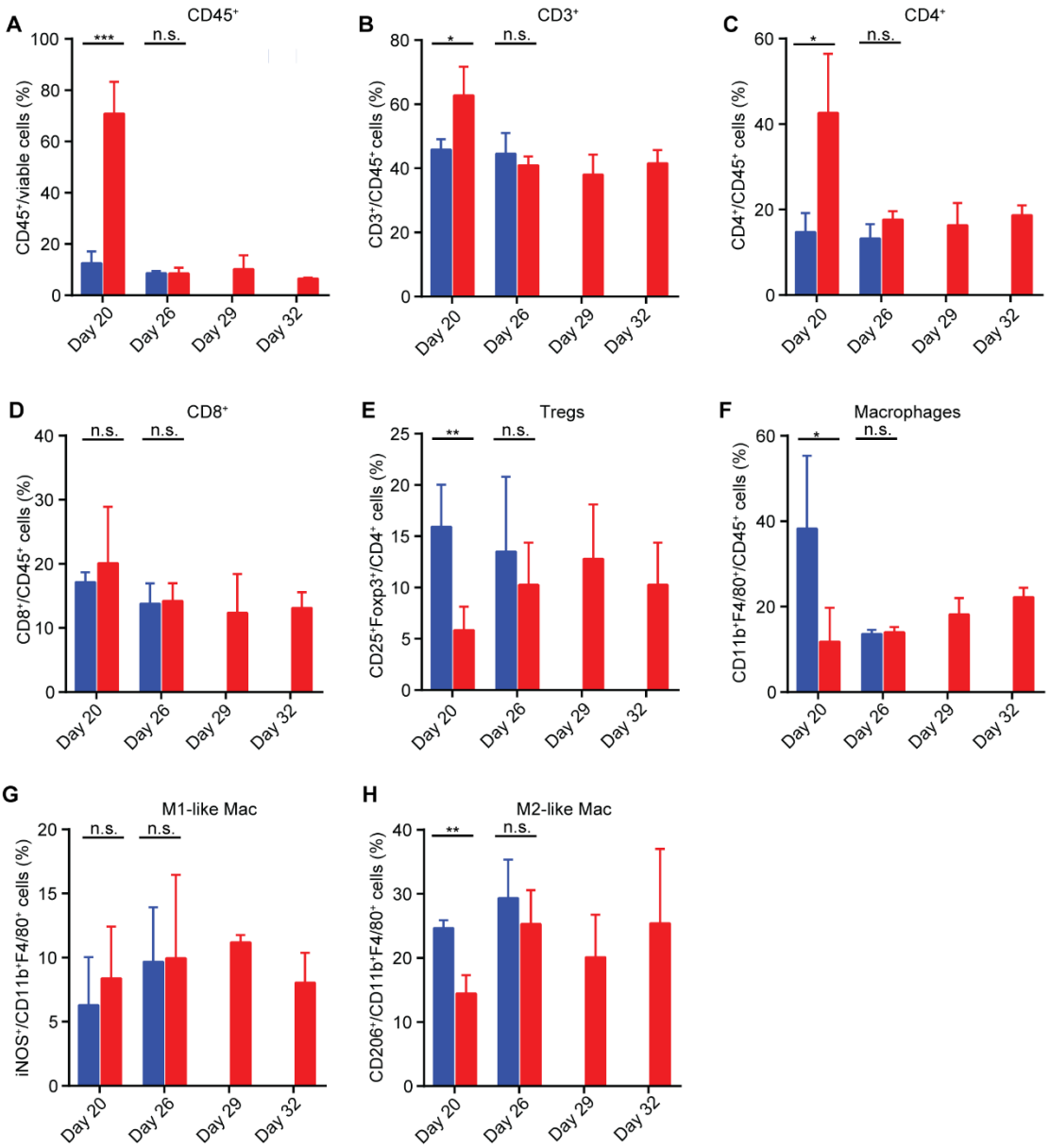

Figure S6. Components of Immune Cells in PDAC Upon Treatment Withdrawal.

Tumor-bearing mice were treated with PBS (blue bars) or oHSV (red bars) as described in Materials and Methods. The fractions of indicated category of cells were monitored on days 20, 26, 29, and 32 by flow cytometry analysis, and presented as percentages as labeled on the vertical axis (n=3). On days 29 and 32, no PBS treated data were available as the tumor size surpassed 2,000 mm^3 and mice were executed. Data were presented as mean values ± standard deviations, and statistics were analyzed using Student's *t*-test (n.s., no significant, *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$).