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Supplemental Information

Oncolytic vaccinia virus induces a novel phenotype of $CD8^+$ effector T cells characterized by high ICOS expression

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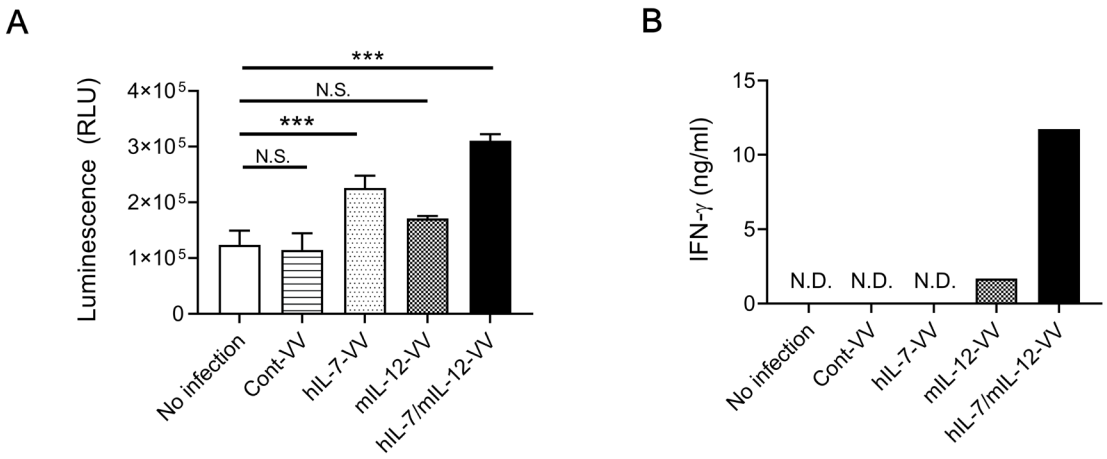
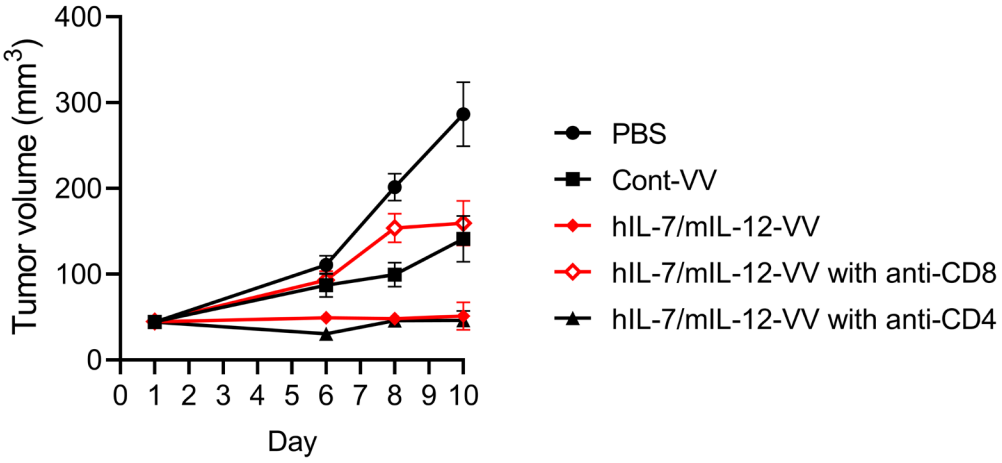
Supplementary Figure S1. Human IL-7 and murine IL-12 secreted by hIL-7/mIL-12-VV-infected cells stimulate murine immune cells.



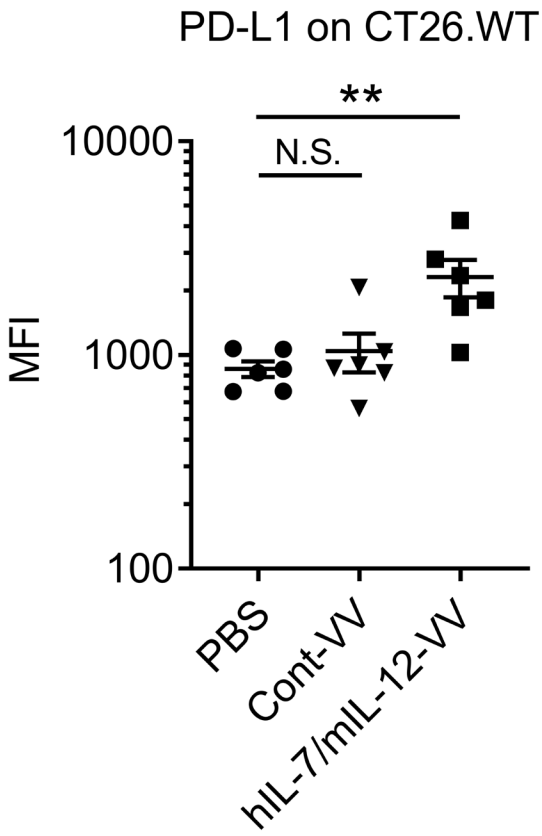
(A) Culture supernatants of A549 cells infected with recombinant viruses at a multiplicity of infection (MOI) = 1.0 were collected and used to stimulate murine splenocytes for three days. Luminescence measured using the CellTiter-Glo Assay which is proportional to the number of splenocytes. Data are mean ± SEM. RLU, relative luminescence unit. (B) Secretion of murine IFN-γ from splenocytes as in (A). N.D., not detected. ***P < 0.001 by Dunnet's multiple comparisons test. N.S., not significant.

Supplementary Figure S2. Depletion of CD8$^+$ T cells suppresses antitumor efficacy of hIL-7/mIL-12-VV.



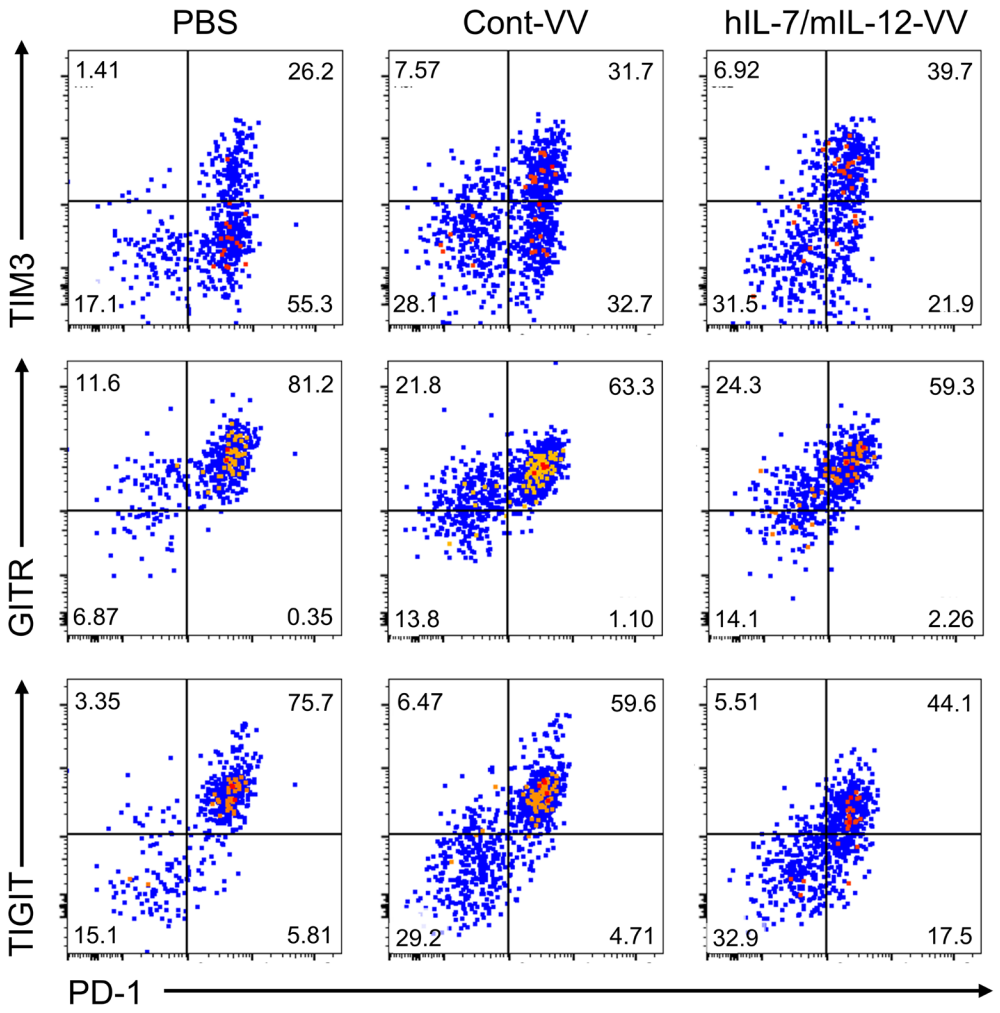
CT26.WT tumors were intratumorally treated with Cont-VV or hIL-7/mIL-12-VV with or without depletion of CD8$^+$ T cells or CD4$^+$ T cells. Tumor growth is shown. n = 6 per group. Mean ± SEM is shown.

Supplementary Figure S3. Intratumoral treatment with hIL-7/mIL-12-VV, but not Cont-VV, induces PD-L1 expression in CT26.WT tumors.



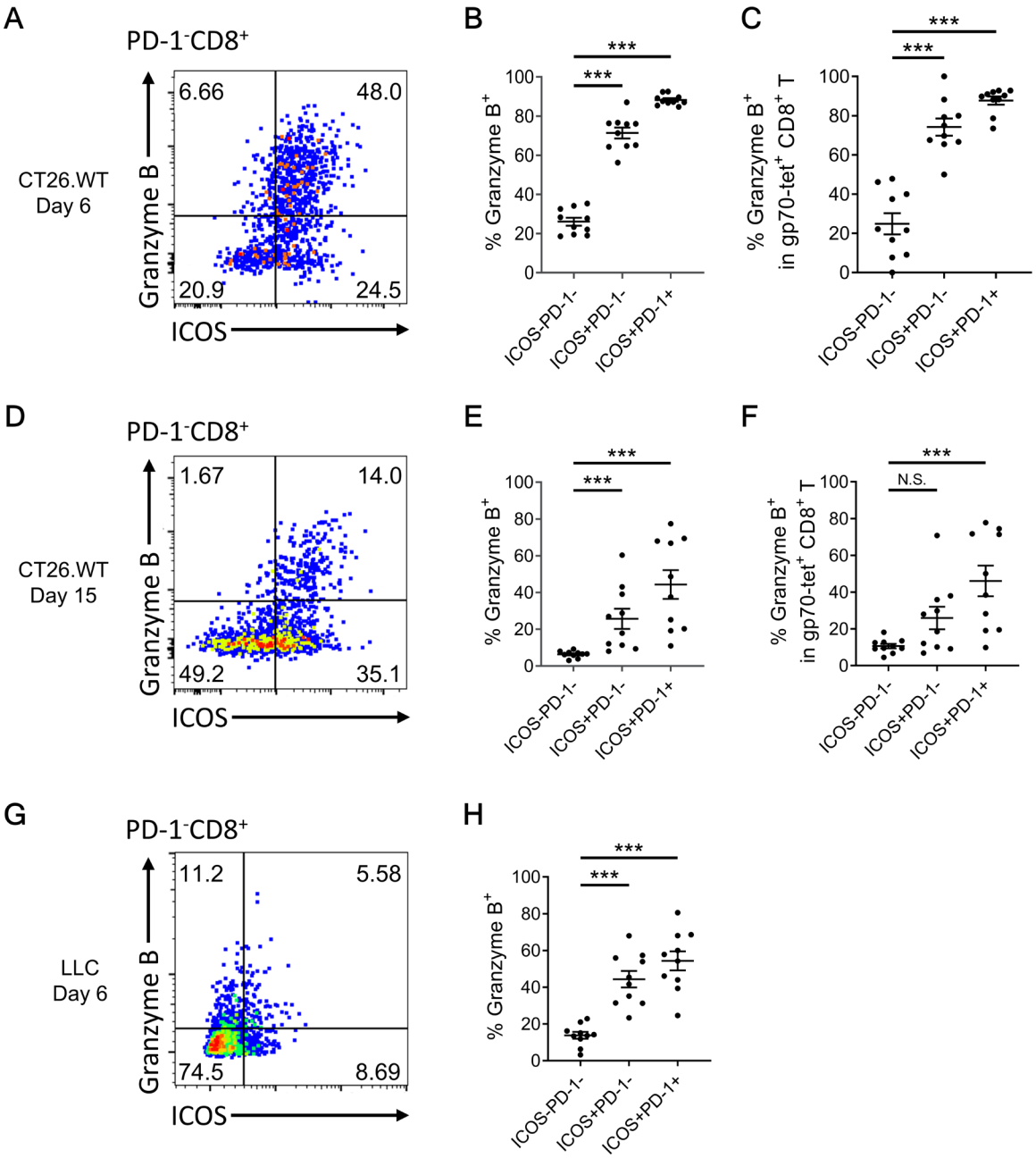
CT26.WT-bearing mice were intratumorally treated with Cont-VV or hIL-7/mIL-12-VV. Seven days after treatment, tumors were collected, and expression of PD-L1 in CT26.WT cells were assessed by flow cytometry (n = 6). Mean ± SEM is shown. $^{**}P < 0.01$ by Mann-Whitney *U* test. N.S., not significant.

Supplementary Figure S4. Representative plots of PD-1, GITR, TIGIT and Tim-3 expression in intratumoral CD8$^+$ T cells.



Mice bearing CT26.WT tumors were intratumorally treated with PBS, 2 × 10^7 pfu of Cont-VV or 2 × 10^7 pfu of hIL-7/mIL-12-VV. Six days after treatment, tumor-infiltrating CD8$^+$ T cells were analyzed by flow cytometry as described in Fig. 2.

Supplementary Figure S5. ICOS$^+$PD-1$^-$CD8$^+$ T cells induced by hIL-7/mIL-12-VV show granzyme B expression.



(A-F) CT26.WT-bearing BALB/c mice were intratumorally treated with hIL-7/mIL-12-VV as described in Fig. 2, and tumors were collected six (A-C) or fifteen (D-F) days after treatment (n =

10 per group). (A and D) Representative plots of granzyme B and ICOS expression in intratumoral PD-1$^-$CD8$^+$ T cells. (B and E) Expression of granzyme B in intratumoral ICOS$^-$PD-1$^-$CD8$^+$ T cells, ICOS$^+$PD-1$^-$CD8$^+$ T cells and ICOS$^+$PD-1$^+$CD8$^+$ T cells. (C and F) Expression of granzyme B in gp70-tetramer$^+$ tumor antigen-specific CD8$^+$ T cells. (G and H) LLC tumors treated with Cont-VV were collected six days after treatment, and tumor-infiltrating CD8$^+$ T cells were analyzed (n = 10). (G) Representative plots of granzyme B and ICOS expression in intratumoral PD-1$^-$CD8$^+$ T cells. (H) Expression of granzyme B in each indicated subset. Mean ± SEM is shown. ***$P < 0.001$ by Mann-Whitney *U* test. N.S., not significant.