Oncolytic vaccinia virus delivering tethered IL-12 enhances antitumor effects with improved safety

Yan Ge[1,3,#], Haiyan Wang[1,3,#], Jinghua Ren[1,4], Weilin Liu[1,5], Lingjuan Chen[1,4], Hongqi Chen[1,6], Junjie Ye[1,7], Enyong Dai[1,8], Congrong Ma[1,2], Songguang Ju[3], Z. Sheng Guo[1,2], Zuqiang Liu[1,2]*, and David L. Bartlett[1,2]*

[1]Department of Surgery, University of Pittsburgh School of Medicine; [2]UPMC Hillman Cancer Center, Pittsburgh, PA 15213, USA; [3]Department of Immunology, School of Biology and Basic Medical Sciences, Medical College, Soochow University, Suzhou, Jiangsu Province, China; [4]Cancer Center, Union Hospital, Huazhong University of Science and Technology, Wuhan, Hubei Province, China; [5]Xiangya School of Medicine, Central South University, Changsha, Hunan Province, China; [6]Shanghai Jiao Tong University Affiliated Sixth People's Hospital, Shanghai, China; [7]Department of Cancer Center, Renmin Hospital of Wuhan University, Wuhan, Hubei Province, China, [8]Department of Oncology and Hematology, China-Japan Union Hospital of Jilin University, Changchun, Jilin Province, China

Running title: Oncolytic virus delivering tethered IL-12 safe and effective

# Y. Ge and H. Wang contributed equally to this paper.

*Corresponding authors:

David L. Bartlett, MD
E-mail: Bartlettdl@upmc.edu

Zuqiang Liu, PhD
E-mail: liuz@upmc.edu

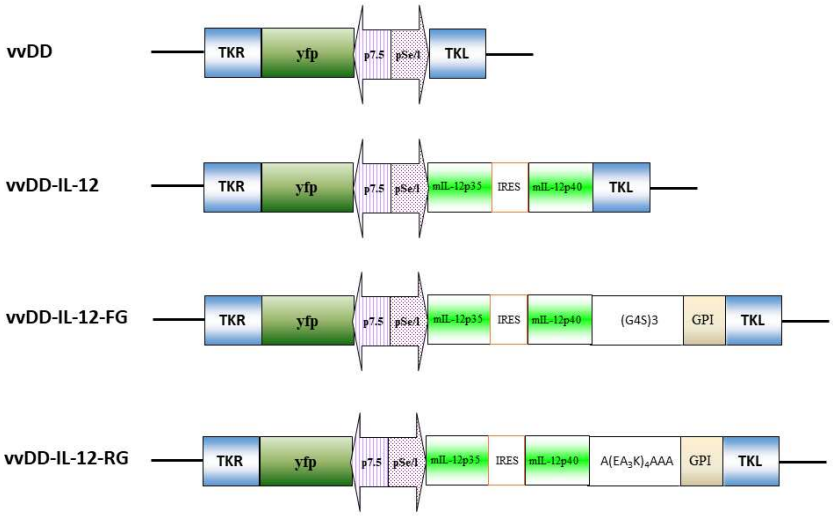

Figure S1. Schematic diagram of viral IL-12 variants. vvDD-IL-12, vvDD-IL-12-FG, and vvDD-IL-12-RG were generated by homologous recombination of murine *IL-12* variants into the *tk* locus of vaccinia viral genome, carrying secreted IL-12, IL-12-flexible linker $(G_4S)_3$-GPI anchor sequence amplified from human CD16b, and IL-12-rigid linker $A(EA_3K)_4AAA$-GPI anchor sequence amplified from human CD16b, respectively.

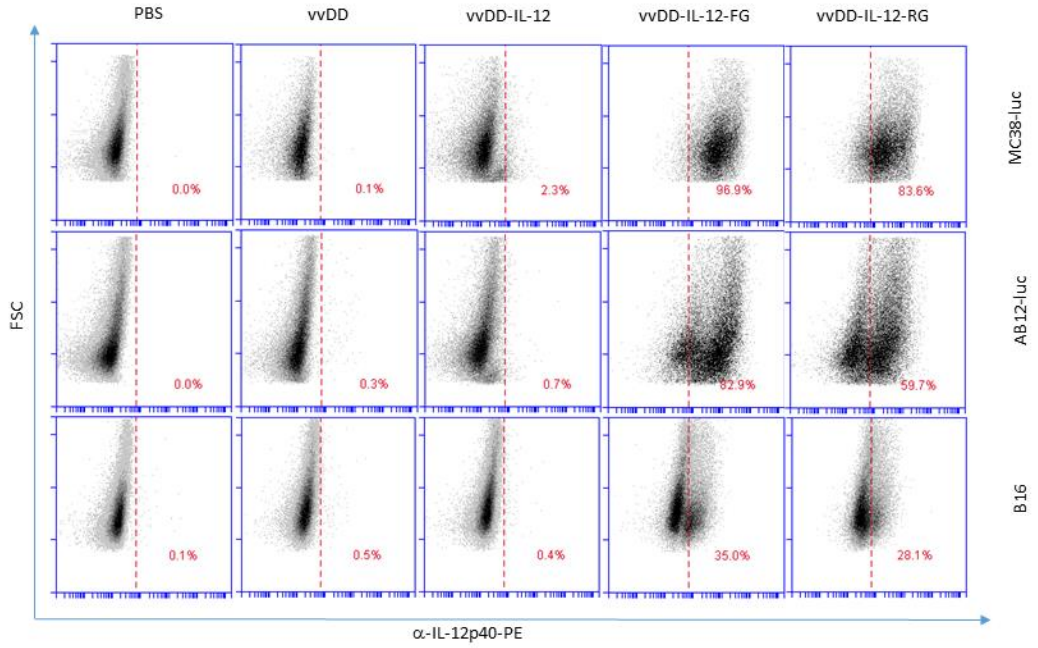

Figure S2. Viral delivered IL-12 expression in tumor cells. Tumor cell MC38-luc (3×10^5 cells), B16 (2×10^5 cells), or AB12-luc (3×10^5 cells) were mock-infected or infected with vvDD, vvDD-IL-12, vvDD-IL-12-FG, and vvDD-IL-12-RG at a MOI of 1. The cell pellets were harvested 24 hours post-infection to measure membrane-bound IL-12 using flow cytometry (cell surface staining).

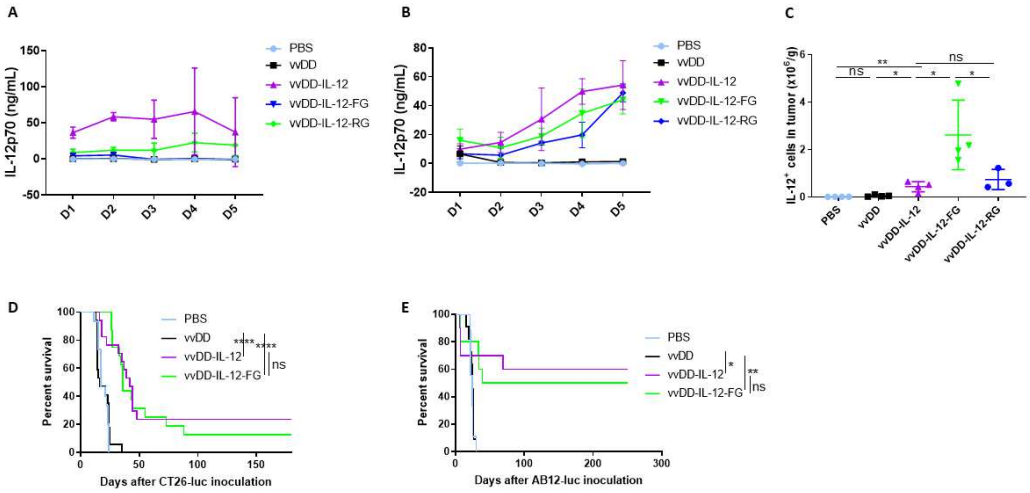

Figure S3. vvDD-IL-12-FG treatment produces tethered IL-12 in tumors and is safe and effective in therapeutic tumor models. B6 mice were i.p. inoculated with 5×10^5 MC38-luc cells and treated with PBS, vvDD, vvDD-IL-12, vvDD-IL-12-FG, or vvDD-IL-12-RG at 5×10^8 PFU/mouse nine days post-tumor inoculation (n=3~5). Sera were collected daily until day 5 to measure the amount of IL-12 (**A**) and IFN-γ in sera (**B**). The mice treated above were sacrificed at day 5 to measure IL-12 membrane association in tumor using flow cytometry (**C**). BalB/c mice were i.p. inoculated with 4×10^5 CT26-luc (**D**) or AB12-luc cells (**E**), respectively, and treated with PBS, vvDD, vvDD-IL-12, or vvDD-IL-12-FG at 2×10^8 PFU/mouse five days post-tumor inoculation and a log-rank (Mantel-Cox) test was used to compare survival rates between these two tumor models. * P<0.05; ** P<0.01; *** P<0.001; and **** P<0.0001. ns: not significant.

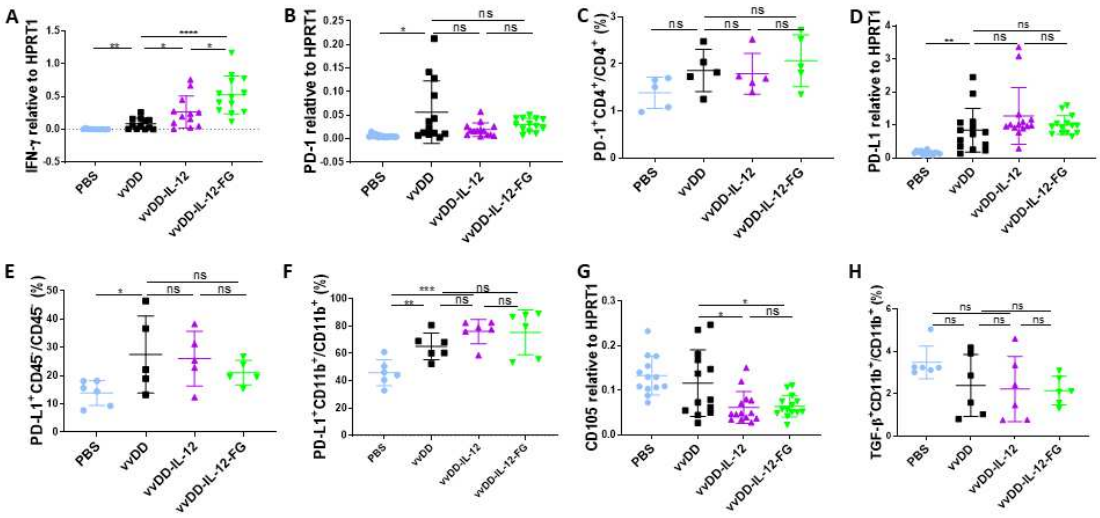

Figure S4. IL-12-variant treatments modify the tumor microenvironment. B6 mice were inoculated i.p. with 5×10^5 MC38-luc cells and treated with PBS, vvDD, vvDD-IL-12, or vvDD-IL-12-FG at 2×10^8 PFU/mouse nine days post-tumor inoculation. Tumor-bearing mice were sacrificed five days post-treatment and primary tumors were collected and analyzed using RT-qPCR to determine IFN-γ (**A**), PD-1 (**B**), PD-L1 (**D**) and CD105 (**G**), using flow cytometry to determine PD-1$^+$CD4$^+$ (**C**), PD-L1$^+$CD45$^-$ (**E**), PD-L1$^+$CD11b$^+$ (**F**) and TGF-β$^+$CD11b$^+$ (**H**) cells. * P<0.05; ** P<0.01; *** P<0.001; and **** P<0.0001. ns: not significant.

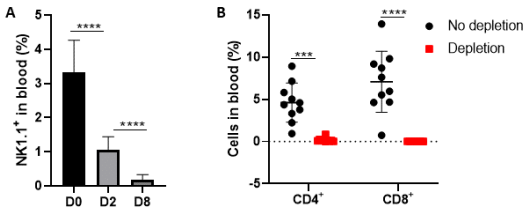

Figure S5. Antibodies can deplete relative cell population efficiently *in vivo*. B6 mice were i.p. inoculated with 5×10^5 MC38-luc cells and treated with α-CD8 Ab (250 μg per injection), α-CD4 Ab (150 μg per injection), PK136 (300 μg per injection) as shown in Fig. 3 P. Blood were collected from mouse tail vein and stained to monitor NK1.1+ cells at day 2 and day 8 after last antibody injection (**A**), $CD4^+$ T cells 3 days after last antibody injection and $CD8^+$ T cells 5 days after last antibody injection (**B**) by flow cytometry, respectively. * $P<0.05$; ** $P<0.01$; *** $P<0.001$; and **** $P<0.0001$. ns: not significant.