oHSV	Insertion	Mouse model	Tumor line	Tumor source
			A20	Murine B lymphoma
T1012G	GFP	Syngeneic mice		
			MFC	Murine forestomach carcinoma
T2850	Murine IL-12	Syngeneic mice	B16	Murine melanoma
			A20	Murine B lymphoma
			MFC	Murine forestomach carcinoma
			1 ( 2 2 2	
			MC38	Murine colon cancer
T3855	Murine IL-12	Syngeneic	MC38	Murine colon cancer
	Murine PD-1 Ab			
			MFC	Murine forestomach carcinoma
			0.007	
			SCC/	Murine head and neck squamous cell
				carcinoma
			<b>B</b> 16	Murino molonomo
			DIO	
			A20	Murine B lymphoma
T3011	Human IL-12	Human PD-1	B16	Murine melanoma
	Human PD-1 Ab	Syngeneic mice		
		Nude mice	KYSE30	Human esophageal squamous
				cell carcinoma

## Supplementary Table 1. List of oHSVs and tumors tested in these studies

List of recombinants and the inserts are shown in columns 1 and 2. The protocols for construction of recombinant viruses produced in our laboratories have been extensively documented elsewhere [33,34]. Sources of the ORFs encoding human and murine anti PD-1 antibodies and IL-12 are listed in *Materials and Methods*.

### **Supplementary Table 2.**

# ELISA detection of expression of IL-12 or PD-1 Ab from T3011, T2850 and T3855 infected cell culture medium

Prod	luction	of	IL-1	2
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- 11017	IL-12	Mean	
0H5V	concen (pg/	+/-SD	
T2011	289.91	293.76	291.83
13011			+/-2.72
T2850	303.26	302.18	302.72
12050			+/-0.38
T2855	664.95	655 24	660.1
13033		055.24	+/-3.44
Mock	U	U	U

#### **Production of PD-1 Ab**

HOM	PD-	Mean	
OHSV	concen (pg/	+/-SD	
T2011	1146.76	1142 42	1144.6
13011		1142.45	+/-3.06
T2850	U	U	U
T2855	1739.07	1752 17	1745.62
13055		1732.17	+/-9.26
Mock	U	U	U

\*U: Undetectable

Vero cells grown in T25 flasks containing  $2x10^6$  cells were mock infected (Mock) or exposed to 1 pfu of T3011, T2850, or T3855 per cell for 1 h. The inoculum was then replaced with fresh medium. The cell culture media (4 ml per flask) were harvested at 48 h after infection. The accumulated levels of human and mouse IL-12 and PD-1 antibodies were analyzed by ELISA assay described in *Materials and Methods*. The amounts of IL-12 and PD-1 Ab were calculated based on a standard curve generated with purified IL-12 or PD-1 antibodies.

#### **Supplementary Figure 1.**

B. Exosomes size distribution



#### A. Exosome markers

**Supplementary Figure 1:** Characterization of exosome carrying miR- CTLA-4. HEp-2 cells seeded in T150 flask were transfected with 10 µg of the miR-CTLA-4 plasmid No. 3 or plasmid expresses non-target miRNA (NT) [32] then incubated in serum free medium. After 48 h the medium was collected and the exosomes were purified as described in Materials and Methods. The purified exosomes were subjected to 2 series of analyses. First (PanelA) equal amounts of cells in which the exosomes were produced and equal amounts of exosomes were solubilized, subjected to electrophoresis in a denaturing gel were probed with antibodies to CD9, Flotilin-1 and Calnexin. Typically, the purified exosomes contained CD9, Flotilin-1 but lacked Calnexin. The size distributions of exosomes (Panel B) produced by transfected cells were done as described in detail elsewhere [32].