

Cell line	JX-594	SJ-607
A549	0.00287	0.00162
MDA-MB-231	0.04784	0.04282
HCT-116	0.07800	0.03120
AGS	0.00835	0.00430
SK-MEL-2	0.01201	0.00781
SNU-1214	0.01313	0.01256
NCI-H1975	0.01533	0.01933
SW620	0.28050	0.12556
SNU-333	0.19787	0.09392
SNU-475	0.02437	0.02901

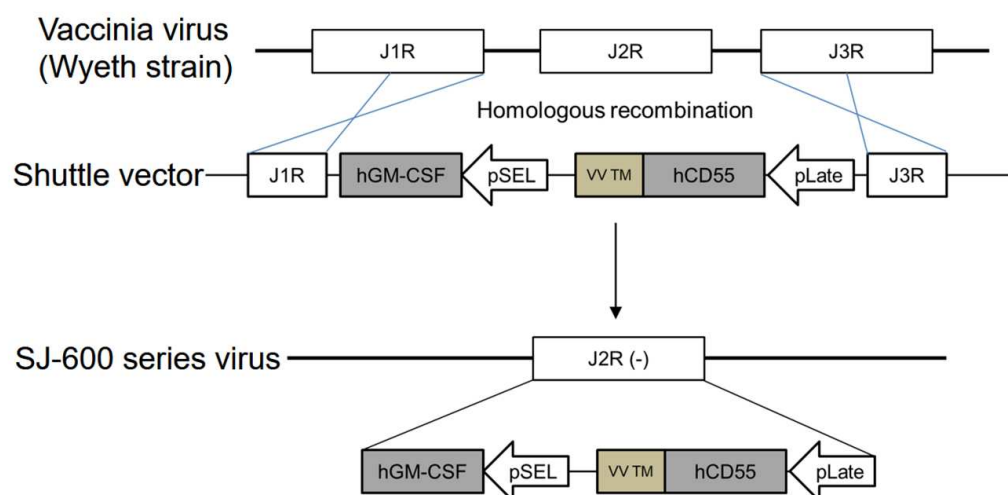
Supplementary Table 1. Mean EC₅₀ values of JX-594 or SJ-607 in 10 cancer cell lines. The *in vitro* cytotoxicities of JX-594 and SJ-607 were examined in various human cancer cell lines, and cell viabilities were measured by CCK-8 assays at 72 h post-infection. The experiments were repeated four times in triplicate and mean EC₅₀ values were calculated. Unit = pfu/cell.

Group		Individual luminescence Intensity (Radiance (p/sec/cm ² /sr)				
		Time After Administration (hr)				
		24	56	96	120	144
SJ-610	Left	2.32E+08	2.06E+08	1.79E+09	3.77E+09	1.42E+09
	Right	1.63E+08	1.74E+08	3.40E+09	4.08E+09	1.33E+09
SJ-607	Left	5.33E+08	6.20E+08	2.01E+09	4.25E+09	1.60E+09
	Right	9.02E+08	1.18E+09	2.85E+09	9.18E+09	2.76E+09

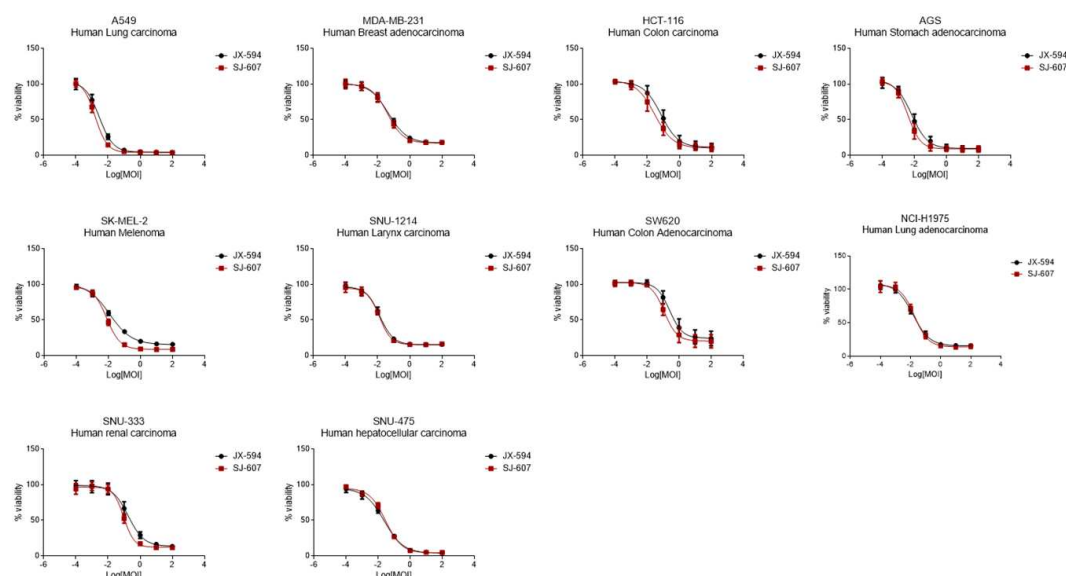
Supplementary Table 2. Luminescence intensity in the tumor site of mice intravenously injected with SJ-607. A single dose of 1 x 10⁶ pfu was intravenously injected into the NSG mice subcutaneously implanted with HCT-116 cells and the presence of virus was analyzed by measuring luciferase activity. Individual data points at 96 h post-injection are shown.

	Mouse No.	Section No.	Tumor area(μm^2)	GFP area(μm^2)	% GFP area /Tumor area
SJ-610	1	2	16,452,180.61	992,652.06	6.03
	1	3	15,687,019.45	767,859.82	4.89
	1	11	33,383,911.32	1,410,090.42	4.22
	1	13	35,718,508.20	1,507,781.28	4.22
	2	3	24,410,610.31	2,370,651.28	9.71
	2	11	32,112,434.21	1,939,754.61	6.04
	3	5	21,746,725.91	1,547,694.78	7.12
	3	7	24,378,942.94	1,768,839.55	7.26
	3	10	31,390,968.56	632,211.03	2.01
	4	2	22,269,320.11	1,308,257.70	5.87
	4	11	32,060,113.67	1,771,717.98	5.53
SJ-607	1	10	18,049,776.93	915,022.33	5.07
	1	16	20,965,619.64	1,434,666.94	6.84
	2	1	34,561,557.38	4,139,688.58	11.98
	2	4	40,790,557.22	4,694,720.32	11.51
	2	5	42,220,602.59	7,708,019.68	18.26
	2	7	46,894,141.87	6,615,545.40	14.11
	3	3	17,527,836.81	1,720,488.51	9.82
	3	10	36,133,201.03	4,310,427.86	11.93
	4	4	27,889,087.75	1,451,047.63	5.20
	4	12	35,751,789.20	2,024,217.87	5.66
	5	3	27,390,404.62	2,838,736.83	10.36
	5	9	38,359,280.23	4,063,904.56	10.59

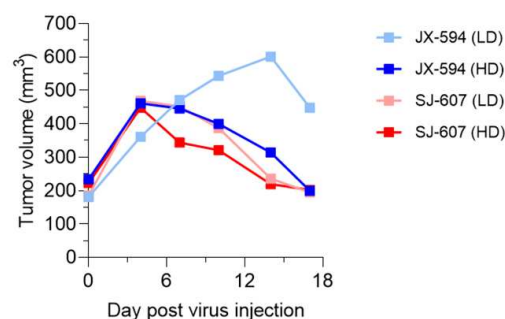
Supplementary Table 3. Mean area density of viruses in tumors of mice intravenously injected with SJ-607. A single dose of 1×10^6 pfu was intravenously injected into the NSG mice subcutaneously implanted with A549 cells. The distributions of viral particles in tumor tissue at 120 h post-injection were analyzed by immunofluorescence. Individual data points are shown.



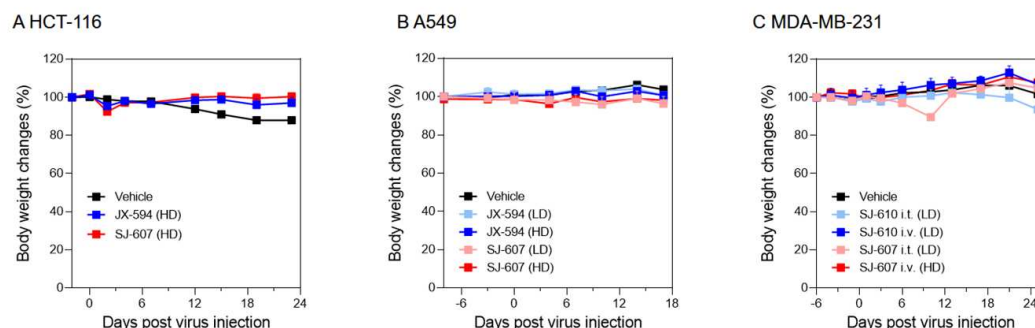
Supplementary Figure 1. Schematic structure of CD55-expressing recombinant vaccinia virus SJ-600 series. CD55-expressing recombinant virus was constructed by insertion of the *CD55* gene fused with the vaccinia virus membrane protein transmembrane domain and *GM-CSF* gene into the *J2R* locus of the Wyeth strain of vaccinia virus through homologous recombination. The *GM-CSF* gene was driven by a synthetic early/late promoter, and the *CD55* gene was driven by a late promoter.



Supplementary Figure 2. *In vitro* cytotoxicity of the SJ-607 recombinant vaccinia virus expressing CD55. Viabilities of 10 human cancer cell lines after inoculation with serially diluted JX-594 or SJ-607 at a MOI of 100 to 0.0001. Cell viabilities were determined by CCK-8 assays at 72 h post-infection. Experiments were conducted four times in triplicate by two independent operators. Graphs show the means \pm standard deviations of four experiments, with uninfected mock control regarded as 100% viability.



Supplementary Figure 3. Antitumor efficacies of intravenously injected SJ-607 in large tumors. A549 human lung cancer cells were subcutaneously implanted into NSG mice and a single dose of recombinant vaccinia virus (high dose, 5×10^6 pfu; low dose, 1×10^6 pfu) was intravenously injected through the tail vein when tumor size was $\sim 200 \text{ mm}^3$. Tumor size was monitored twice weekly.



Supplementary Figure 4. Body weight changes in tumor-bearing mice systemically administered SJ-607. Tumor cells were subcutaneously implanted into NSG mice and a single dose of recombinant vaccinia virus (high dose, 5×10^6 pfu; low dose, 1×10^6 pfu) was intravenously injected through the tail vein when tumor size was approximately 80–120 mm³. (A) Body weight changes in HCT-116 human colon cancer model (high-dose groups only), (B) in A549 human lung cancer model, and (C) in MDA-MB-231 human triple-negative breast cancer model (low-dose groups only).