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Supplemental Information

OvAd1, a Novel, Potent, and Selective Chimeric Oncolytic Virus Developed for Ovarian Cancer by 3D-Directed Evolution

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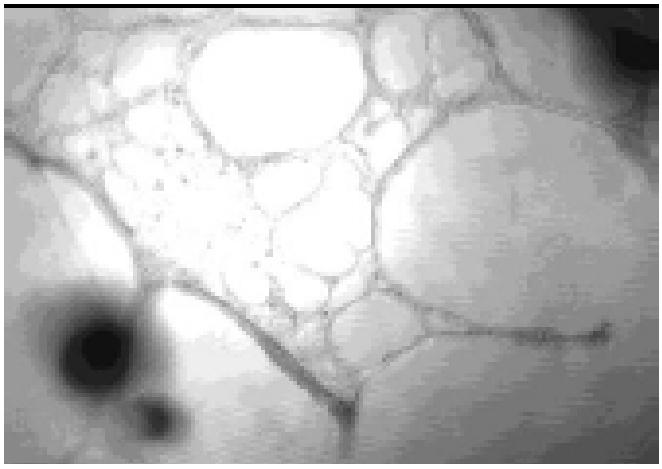
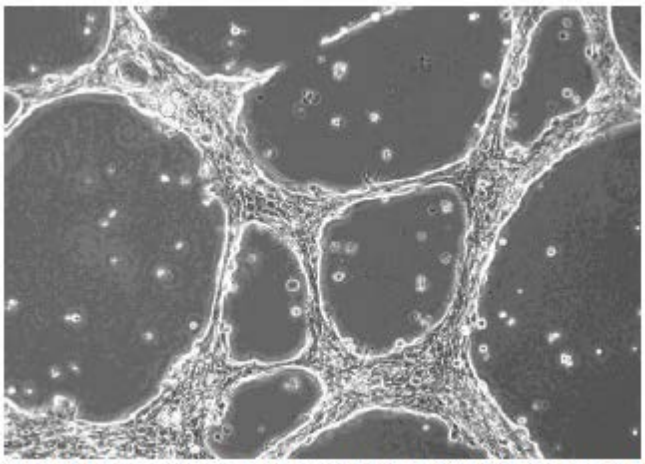
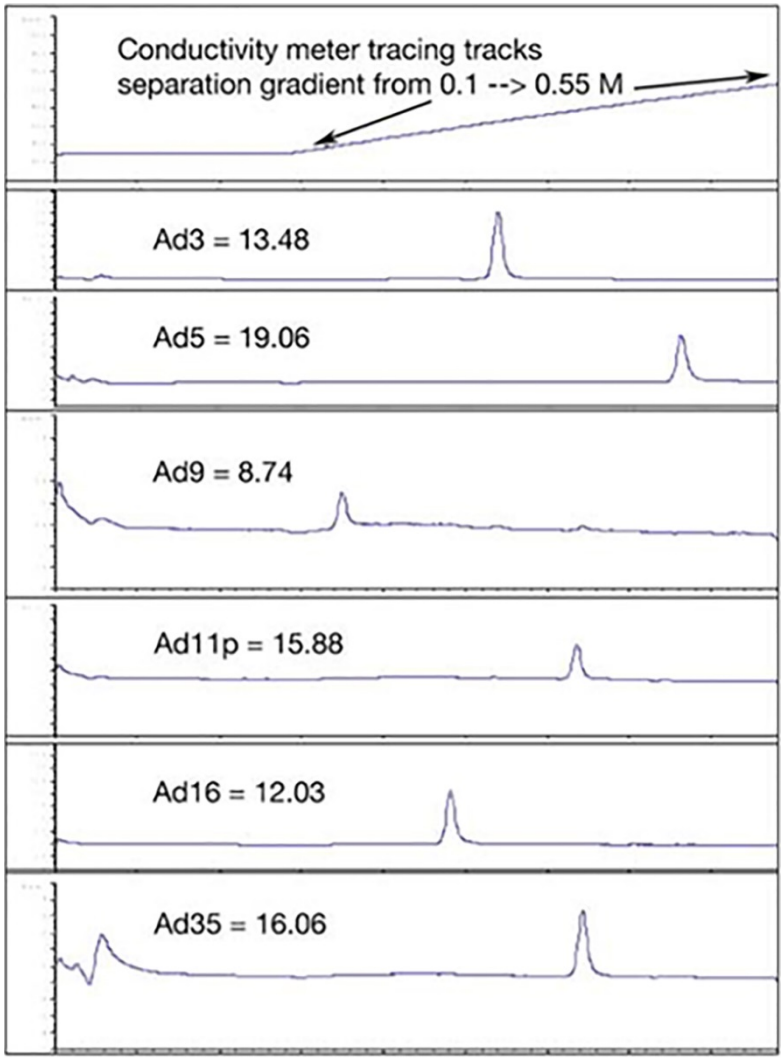


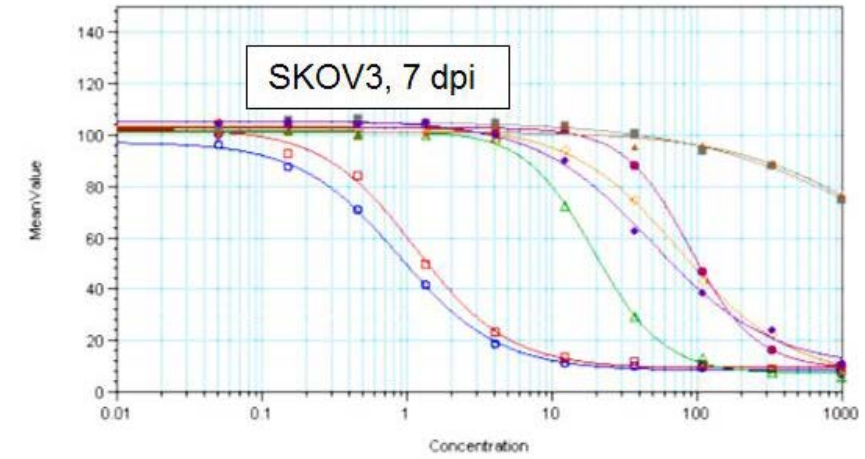


SKOV3 matrigel 1e7 seeded 8-12-03, 2 dps 100x

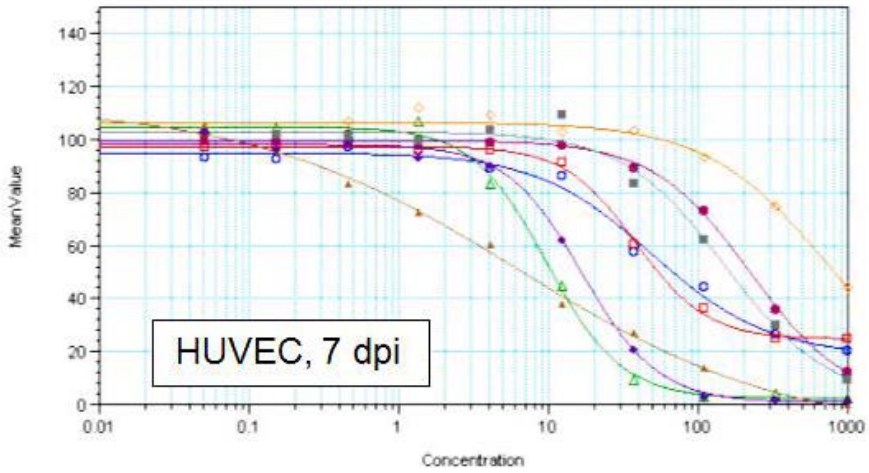
Supplementary Figure 1: Images of SKOV3 soon after seeding onto Matrigel: The first image is focused well-above the surface of the Matrigel in order to reveal the rope-like structures formed by the SKOV3 cells. (The large dark blob at the lower left is a bubble in the Matrigel, quite out of focus). The second image is taken closer to the Matrigel surface, and reveals cellular refraction indicative of the height of the rope-like strands.



Supplementary Figure 2: Below are mAU 260 (y-axis) chromatograms of AIEX-HPLC analyses of pure stocks of adenoviral serotypes. Each purified adenoviral serotype has a distinct AIEX elution position (x-axis=minutes), indicated as number of minutes to elution given the standard separation salt gradient. The position of each narrow peak in the 260 chromatogram is sufficient to both identify the serotype and to quantify the concentration of the stock as viral particles per ml (Figure1 from Kuhn, Gene Therapy, 2007). Chromatograms showing the elution characteristics of additional serotypes are included in Figure1 of Kuhn et al, PLoS One, 2009.



y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
Plot#1 (OvAd1: VP/cell vs %control)	97.304	1.271	0.885	8.262	0.999
Plot#2 (OvAd2: VP/cell vs %control)	102.603	1.351	1.146	9.207	0.997
Plot#3 (ColoAd1: VP/cell vs %control)	101.746	1.808	19.5	7.146	0.999
Plot#4 (Ad3: VP/cell vs %control)	103.55	1.15	77.027	5.53	0.999
Plot#5 (Ad11p: VP/cell vs %control)	103.043	1.9	89.127	9.042	0.998
Plot#6 (ONYX-015: VP/cell vs %control)	105.255	0.697	1411.081	36.968	0.989
Plot#7 (Ad5: VP/cell vs %control)	101.494	0.834	1893.447	33.479	0.945
Plot#8 (Ad35: VP/cell vs %control)	105.698	1.069	50.114	9.404	0.997



y = ((A - D)/(1 + (x/C)^B)) + D:	A	B	C	D	R^2
Plot#1 (OvAd1: VP/cell vs %control)	95.21	1.116	48.926	18.319	0.99
Plot#2 (OvAd2: VP/cell vs %control)	97.605	1.818	38.203	24.579	0.998
Plot#3 (ColoAd1: VP/cell vs %control)	104.897	1.746	9.707	2.227	0.998
Plot#4 (Ad3: VP/cell vs %control)	106.575	1.065	744.663	-1.621	0.98
Plot#5 (Ad11p: VP/cell vs %control)	99.773	1.323	223.393	-0.217	0.998
Plot#6 (ONYX-015: VP/cell vs %control)	103.127	1.266	150.692	1.634	0.986
Plot#7 (Ad5: VP/cell vs %control)	112.926	0.484	6.317	-11.475	0.998
Plot#8 (Ad35: VP/cell vs %control)	98.301	1.709	16.758	0.638	0.997

Supplementary Figure 3: OvAd1 and OvAd2, the products of directed evolution, acquired 2-3 logs more potency on SKOV3 relative to the viruses in the starting pool; however, their potency on HUVEC was limited relative to the starting pool.