OMTO, Volume 9

Supplemental Information

Endogenous Akt Activity Promotes Virus Entry

and Predicts Efficacy of Novel Chimeric

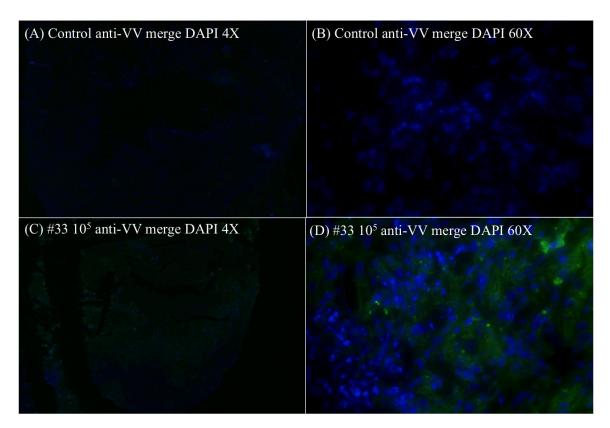
Orthopoxvirus in Triple-Negative Breast Cancer

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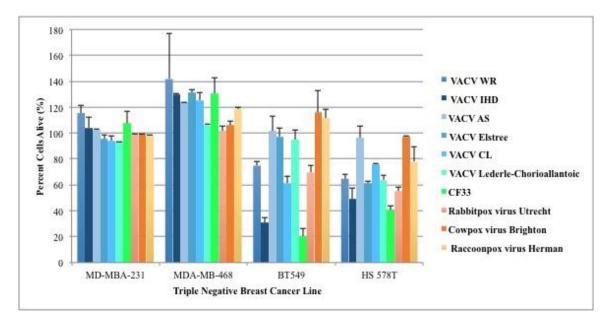
Supplementary Data

Supplemental Figure 1. CF33 infects MDA-MB-468 tumors in vivo.

Immunofluorescent detection of polyclonal antibody against vaccinia virus demonstrates viral infection of MDA-MB-468 xenograft tumor tissue harvested one week after intratumoral CF33 injection. (A) Control tumor, 4X, (B) Control tumor, 60X, (C) Tumor treated with 10⁵ PFU, 4X, (C) Tumor treated with 10⁵ PFU, 60X. (VV: green; DAPI counterstain: blue).



Supplemental Figure 2. High-throughput screening of CF33 against parental viruses. All cells were infected at 0.01 MOI and assayed for cell viability at 96 hours post-infection. While none of the viruses were effective against MD-MBA-231 or MD-MBA-468 at this particular screening MOI, CF33 was more effective than any of the parental viruses against BT549 and Hs578T and was thus selected for further study. Data are presented as means ± standard deviation of the means.



Virus	MD-MBA-231		MDA-MB-468		BT549		HS 578T	
	%		%		%		%	
	Cells	SD	Cells	SD	Cells	SD	Cells	SD
	Alive		Alive		Alive		Alive	
VACV WR	115.46	5.89	141.72	35.53	74.84	3.14	64.75	3.56
VACV IHD	104.11	8.32	129.44	1.04	30.91	3.69	49.18	8.27
VACV AS	102.31	0.92	123.57	0.52	101.56	11.86	95.97	9.80
VACV Elstree	95.36	2.89	131.09	2.33	97.22	6.36	61.42	1.65
VACV CL	94.14	3.70	125.40	5.71	61.40	5.50	75.73	0.76
VACV								
Lederle-								
Chorioallantoic	93.32	0.00	106.51	0.26	95.00	7.62	63.94	3.69
CF33	107.46	9.36	130.35	12.19	20.52	5.81	40.72	3.44
Rabbitpox								
virus Utrecht	99.04	0.69	101.74	3.37	69.56	5.11	55.21	3.05
Cowpox virus								
Brighton	99.04	0.46	105.96	3.11	116.11	17.04	97.32	0.25
Raccoonpox								
virus Herman	98.14	0.35	118.62	1.30	111.61	6.75	77.80	11.84

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