

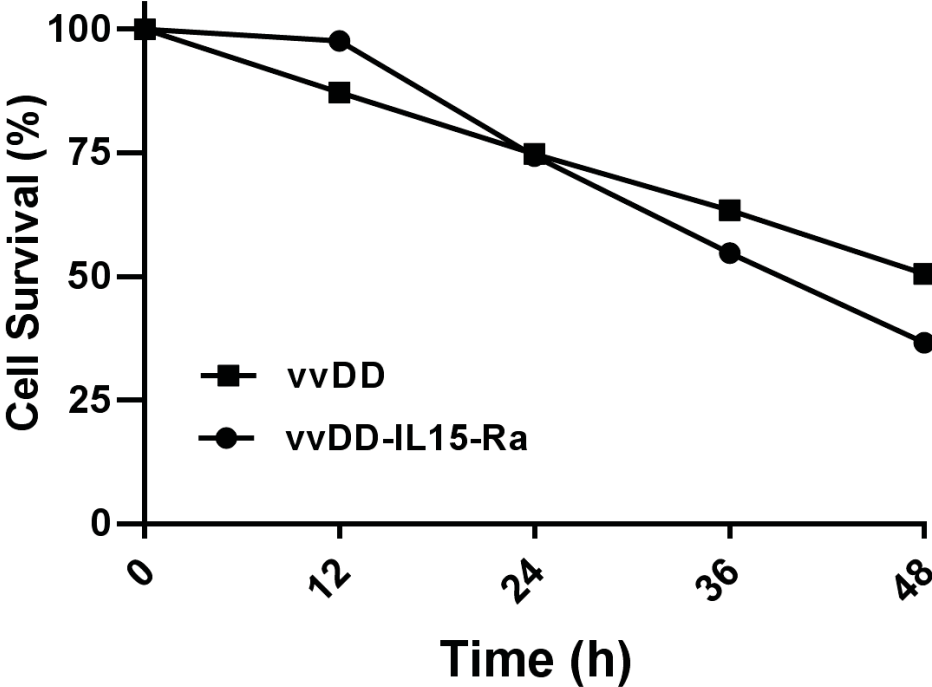
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Supplemental Information

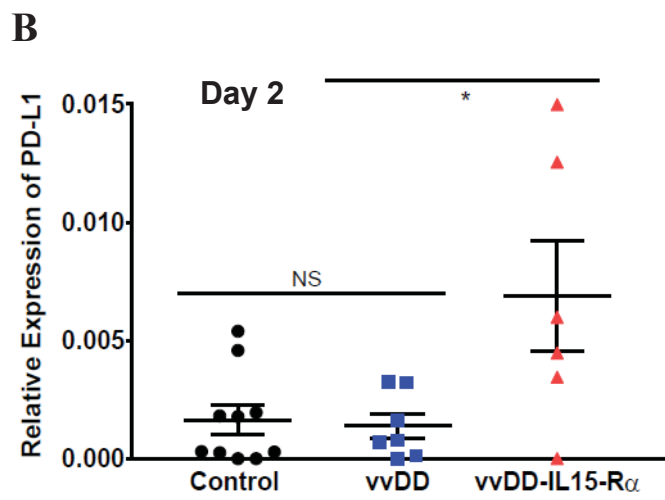
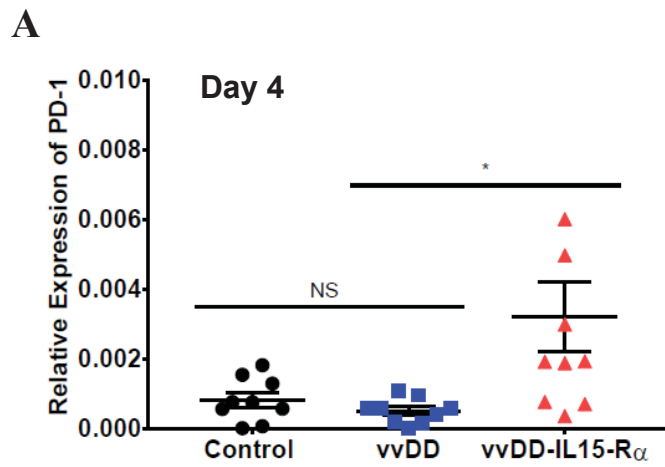
Superagonist IL-15-Armed Oncolytic Virus Elicits Potent Antitumor Immunity and Therapy That Are Enhanced with PD-1 Blockade

Stacy J. Kowalsky, Zuqiang Liu, Mathilde Feist, Sara E. Berkey, Congrong Ma, Roshni Ravindranathan, Enyong Dai, Edward J. Roy, Zong Sheng Guo, and David L. Bartlett

Supplemental Figure 1. The cytotoxicity of oncolytic viruses in MC38 colon cancer cells



Supplemental Figure 2. PD-1 and PD-L1 expression (RT-qPCR)



Supplemental Table 1. Viral therapeutic and survival data by experimental model

§ Survival data not represented graphically

Experiment	Treatment Group	n	Median survival (days)	<i>p</i> vs Control	<i>p</i> vs vvDD	<i>p</i> vs vvDD-IL15-R α
MC38 Tumor Progression/Survival (Fig. 3)	Control	9	18	-	***	***
	vvDD	8	36	***	-	***
	vvDD-IL15-R α	5	> 130	***	***	-
ID8 Tumor Progression/Survival (Fig. 4)	Control	10	46.5	-	****	****
	vvDD	9	87	****	-	*
	vvDD-IL15-R α	10	> 130	****	*	-

Experiment	Treatment Group	n	Median survival (days)	<i>p</i> vs Control	<i>p</i> vs vvDD-IL15-R α	<i>p</i> vs Virus + α -CD8	<i>p</i> vs Virus + α -NK cell	<i>p</i> vs Virus + α -CD4
Selective Immune Depletion (Fig. 5E,F) §	Control	9	18	-	****	****	****	****
	vvDD-IL15-R α	10	> 150	****	-	**	NS	NS
	Virus + α -CD8	10	51.5	****	**	-	**	***
	Virus + α -NK cell	10	> 150	****	NS	**	-	NS
	Virus + α -CD4	10	> 150	****	NS	***	NS	-

Experiment	Treatment Group	n	Median survival (days)	<i>p</i> vs Control	<i>p</i> vs α -PD1	<i>p</i> vs vvDD	<i>p</i> vs vvDD-IL15-R α	<i>p</i> vs vvDD+ α -PD1	<i>p</i> vs vvDD-IL15-R α + α -PD-1
Combination with PD-1 Blockade (Fig. 6B)	Control	7	20	-	*	***	***	***	***
	α -PD1	7	27	*	-	NS	***	**	***
	vvDD	7	31	***	NS	-	**	*	***
	vvDD-IL15-R α	8	40	***	***	**	-	NS	***
	vvDD + α -PD1	8	38.5	***	**	*	NS	-	***
	vvDD-IL15-R α + α -PD1	7	> 200	***	***	***	***	***	-

Supplemental Table 2. RT-qPCR Primers Utilized

Primer	Thermo Fisher Scientific primer	Gene symbol	RefSeq	Translated Protein	
CD8	Mm01182107_g1	CD8a	NM_001081110.2 NM_009857.1	NP_001074579.1 NP_033987.1	
NKp46	Mm00456776_g1	Ncr1	NM_010746.3	NP_034876.2	
NKG2D	Mm00473603_m1	Klrk1	NM_001083322.2 NM_001286018.1 NM_033078.4	NP_001076791.1 NP_001272947.1 NP_149069.1	
PD-1	Mm1285676_m1	Pdcd1	NM_008798.2	NP_03824.1	
PDL-1	Mm00452054_m1	CD274	NM_021893.3	NP_068693.1	
HPRT¶	Mm00446968_m1	Hprt	NM_0135562.2	NP_038584.2	
GAPDH	Mm99999915_g1	Gapdh	NM_00128926.1 NM_008084.3	NP_001276655.1 NP_032110.1	
Custom Primer			Forward Sequence	Reverse Sequence	Reporter sequence
A34R	-	-	GTCTACAGATAATGCGGTTA TCAGTGT	ACTCTCAGATGTCTAGTAT CCGGTCTA	CTGGCTCGTAATTAC
IL15-R α	-	-	Forward Sequence	Reverse Sequence	Hybridization Oligomer
			TGAGGAACGTGCTGTACCTG	GGTCTTCTCCTCCAGCTCCT	GAGAGCGGCTGTAAGGAG TG

1. ¶ house-keeping genes.