

Figure 1. Construction of NDV 73T antigenomic cDNA

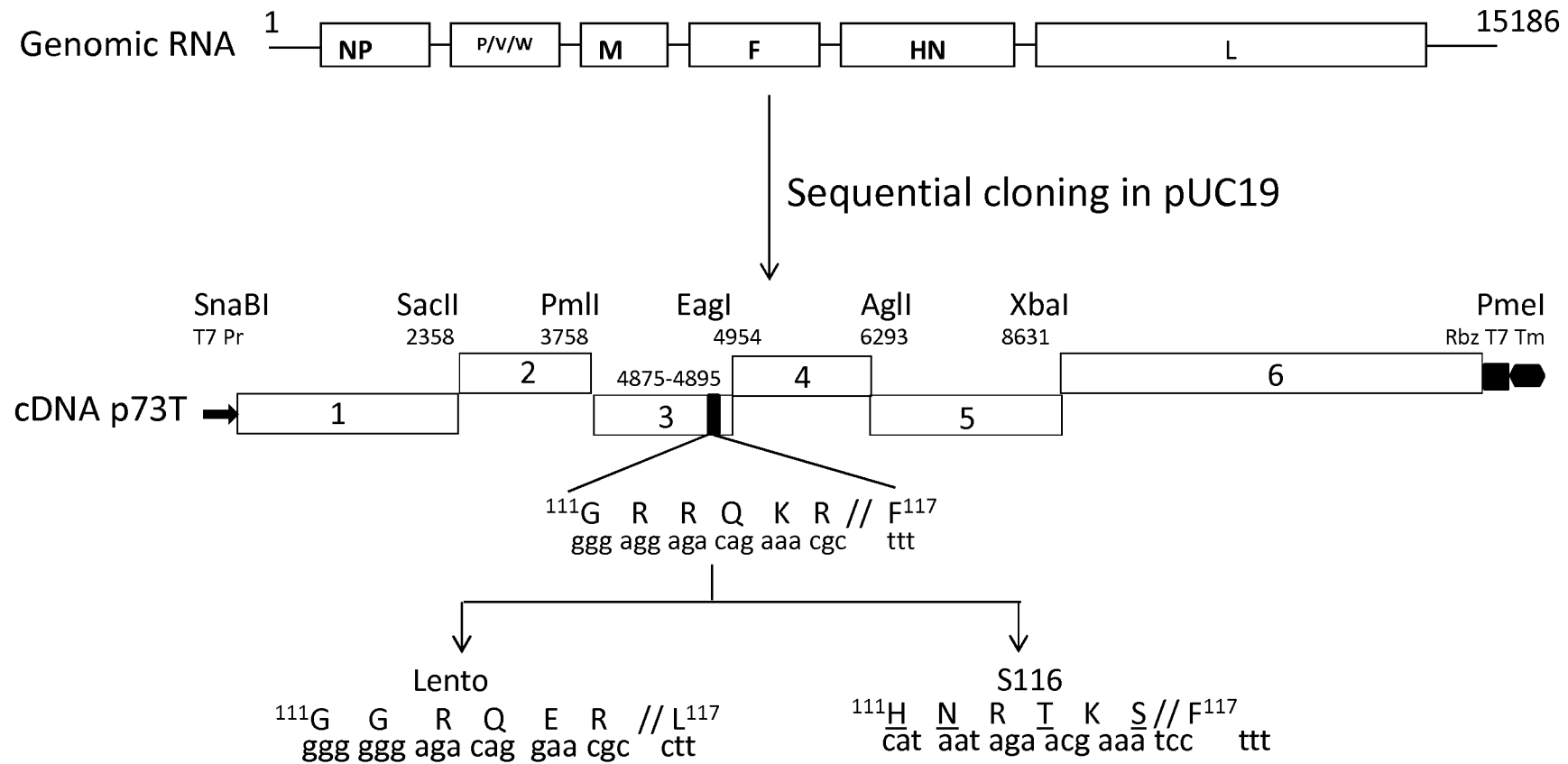


Figure 2. Insertion of transgene cassette(s) into NDV 73T genome

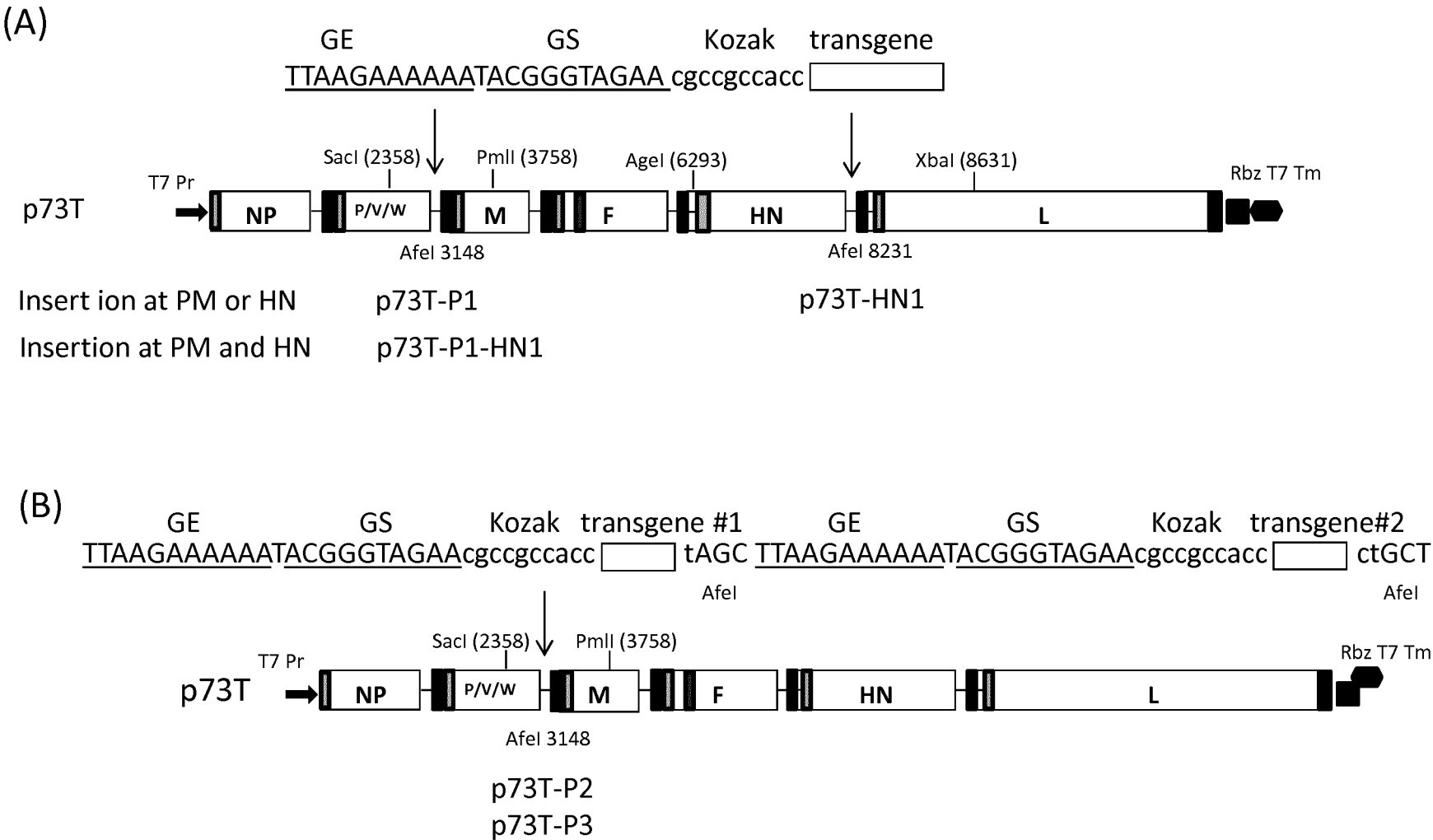
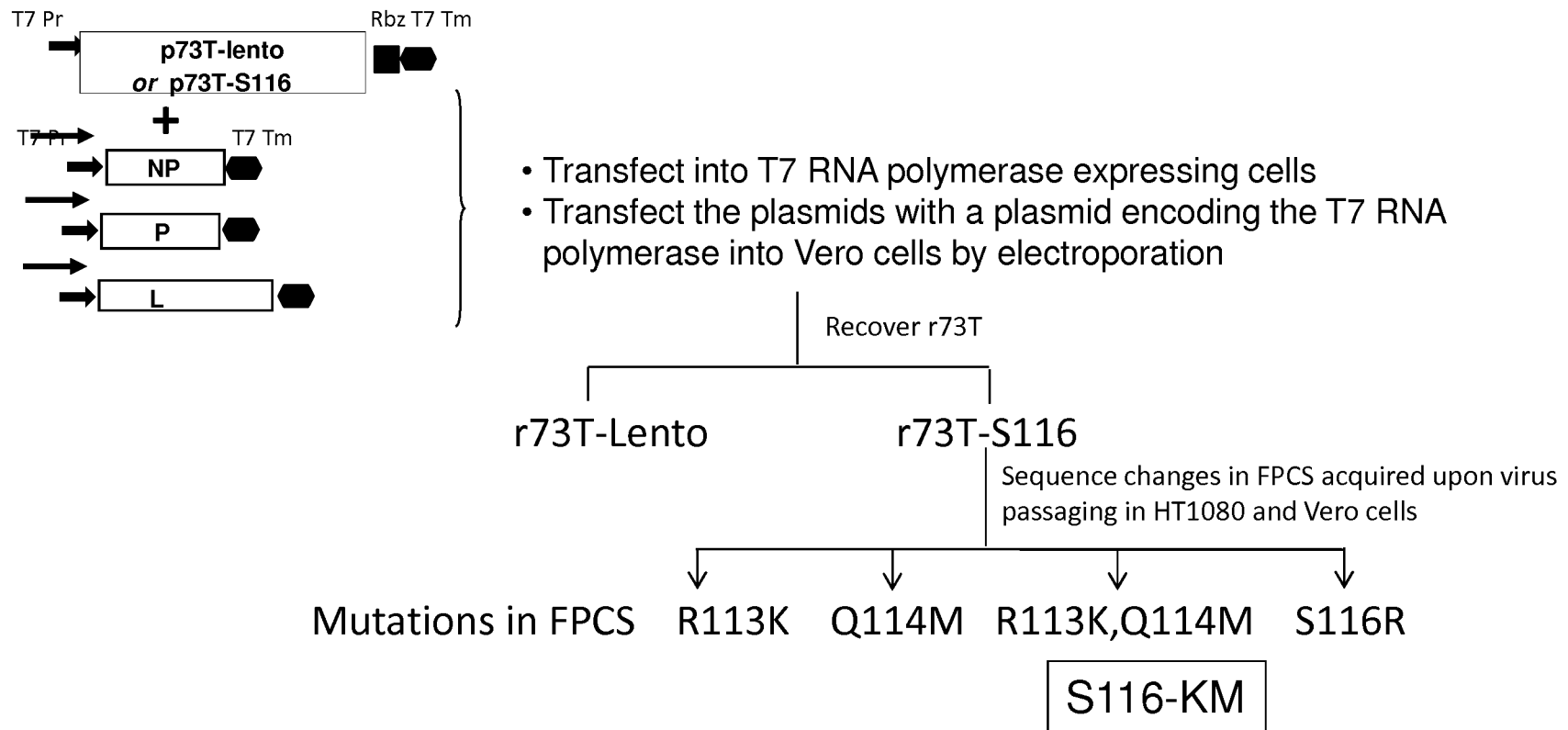


Figure 3A. Method for recovering infectious recombinant NDV strain 73T (r73T) with modified FPCS



Figures 3B and C. Evaluation of F protein cleavage and fusion activity in vitro

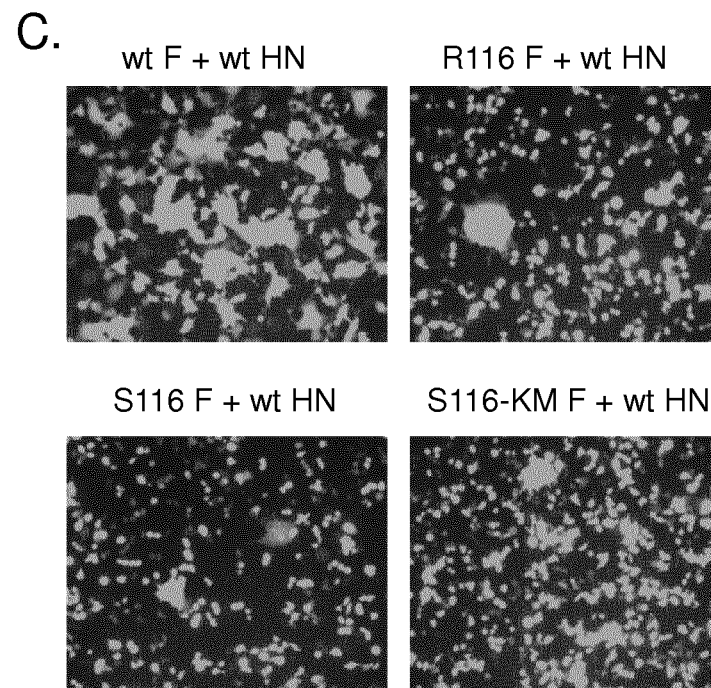
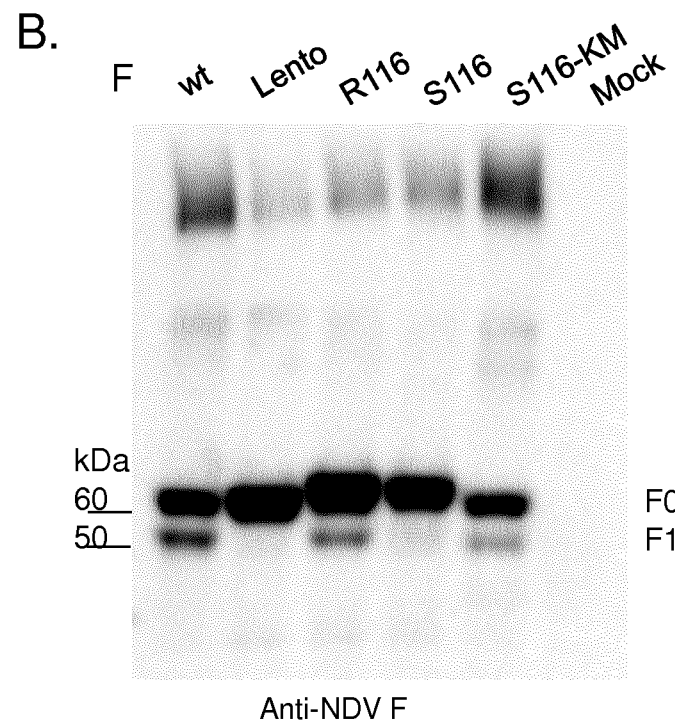


Figure 4. Summary of characteristics of r73T-lento and r73T-S116 derivatives

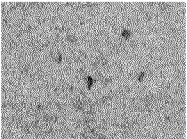
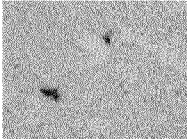
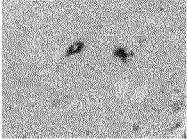
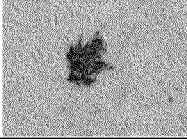
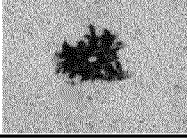
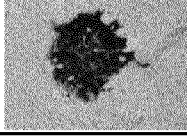
Virus name (73T) ^a	FPCS sequence ^b	Plaque formation ^c	MDT (hrs) ^d	ICPI ^e	Relative HT1080 cell killing ^e	Vero (pfu/ml) ^g	Eggs (pfu/ml) ^h
-lento	¹¹¹ G-G-R-Q-E-R/L-I ¹¹⁸		> 156	0.00	13	7.5E+03	5.7E+08
-S116	¹¹¹ H-N-R-T-K-S/F-I ¹¹⁸		> 156	0.00	31	4.4E+06	4.6E+08
-S116K113	¹¹¹ H-N- <u>K</u> -T-K-S/F-I ¹¹⁸		> 156	ND	29	3.4E+06	3.8E+08
-S116M114	¹¹¹ H-N-R- <u>M</u> -K-S/F-I ¹¹⁸		> 156	ND	31	3.6E+06	3.4E+08
-S116K113M114 (S116-KM)	¹¹¹ H-N- <u>K</u> - <u>M</u> -K-S/F-I ¹¹⁸		> 156	0.00	48	2.4E+06	4.2E+08
-R116	¹¹¹ H-N-R-T-K- <u>R</u> /F-I ¹¹⁸		66	0.65	80	6.2E+06	1.7E+08

Figure 5. Strategies to attenuate r73T-R116 virus virulence in chicken

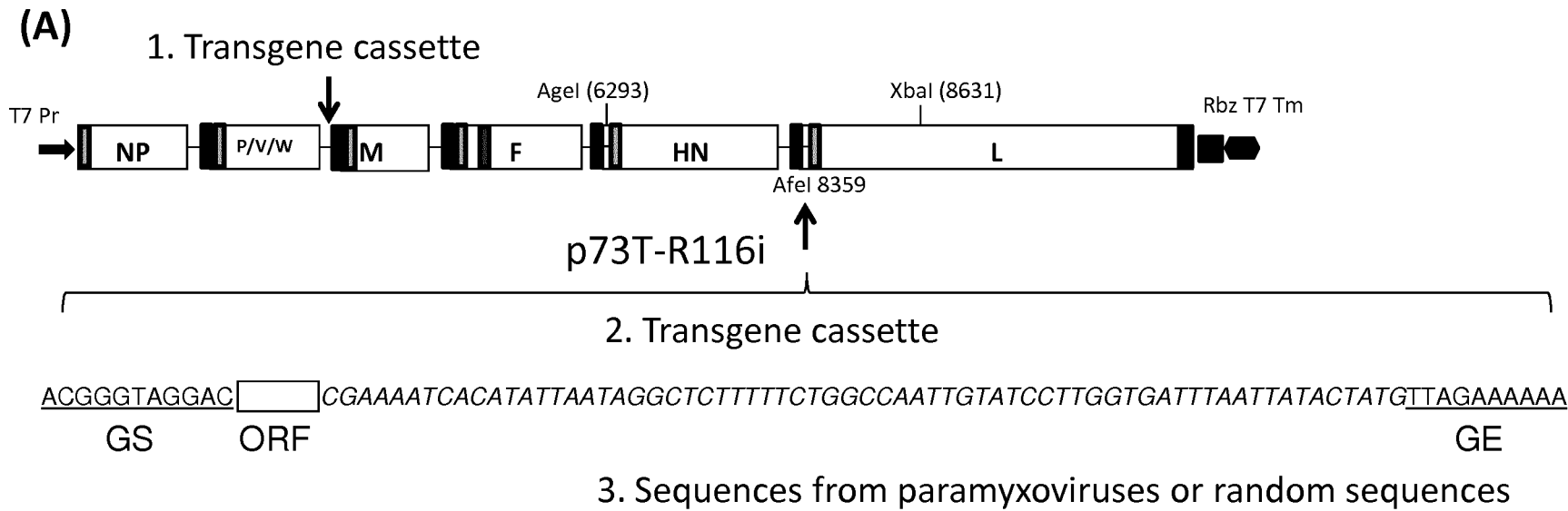


Figure 5. continued

(B) Non-coding sequences inserted at HN-L junction

APMV-1 N 318 nt

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attactcacatgcagctgaagtaggaatgcagtacgtgagcaccacactgggagcagagcgtacacagcagataactaaagaactcaggtagtgggtgcaggcagtattgaccaagacatact
ctcttgggaagggcaaaaacagcaaaggggaggagttgcaaatgtagacatacatggggttgaaagaag

RSV-NP 198 nt

acttctgtcatccagcaaatacgccatccaacggagcacaggagatagtattgatactcctaattatgatgtgcagaaacacatcaataagttatgtggcatgttattaatcacagaagatgcta
cataaattcactgggttaataggtatgttatatgcgatgtctaggttaggaagagaagacaccataaaaat

Random sequence 198 nt

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Random sequence 144 nt

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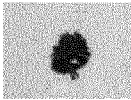
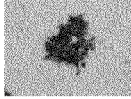
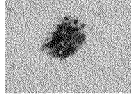
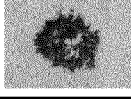
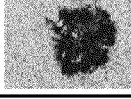
Random sequence 102 nt

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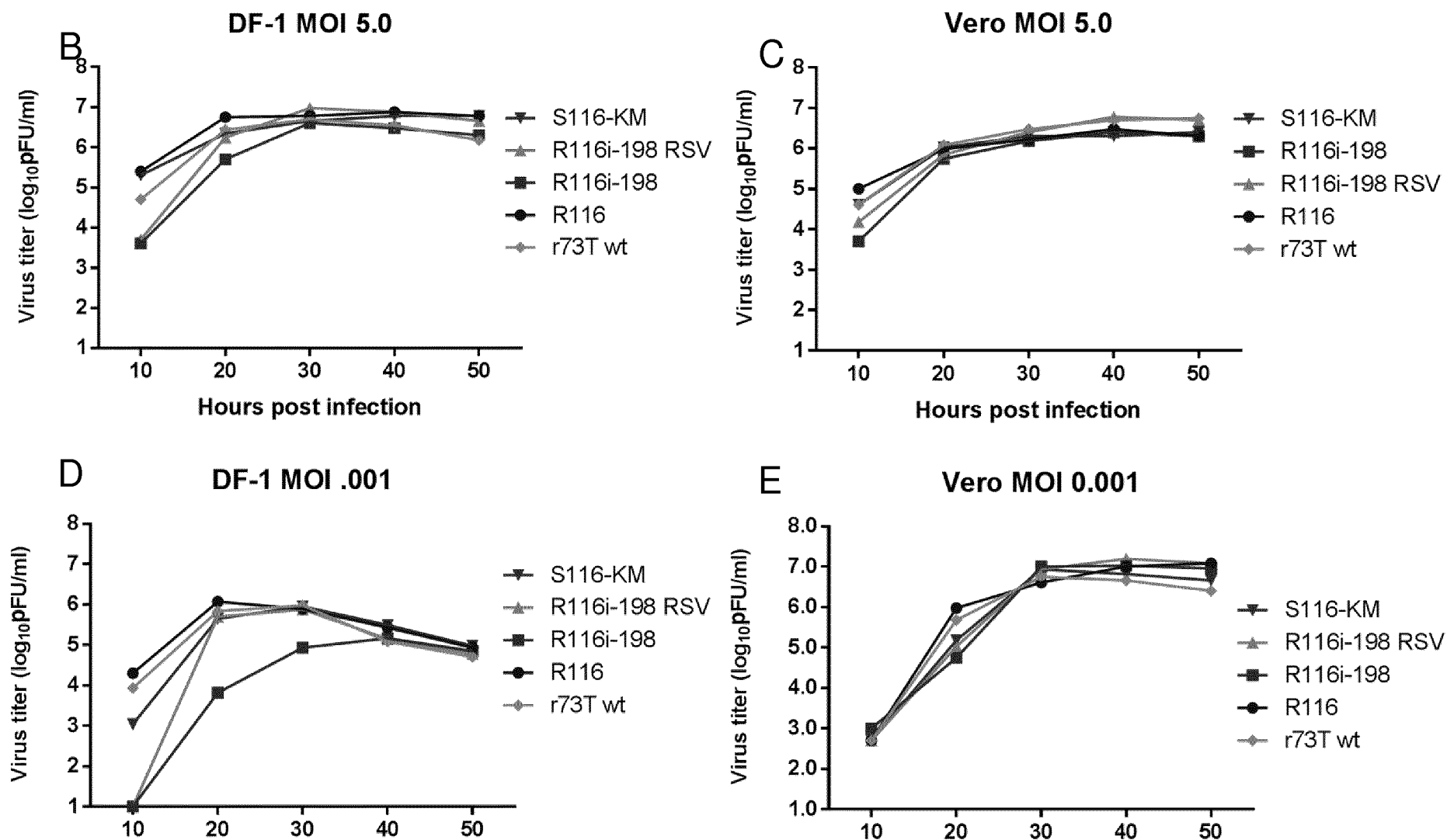
Random sequence 60 nt

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Figure 6A. Summary of characteristics of r73T-R116 derivatives.

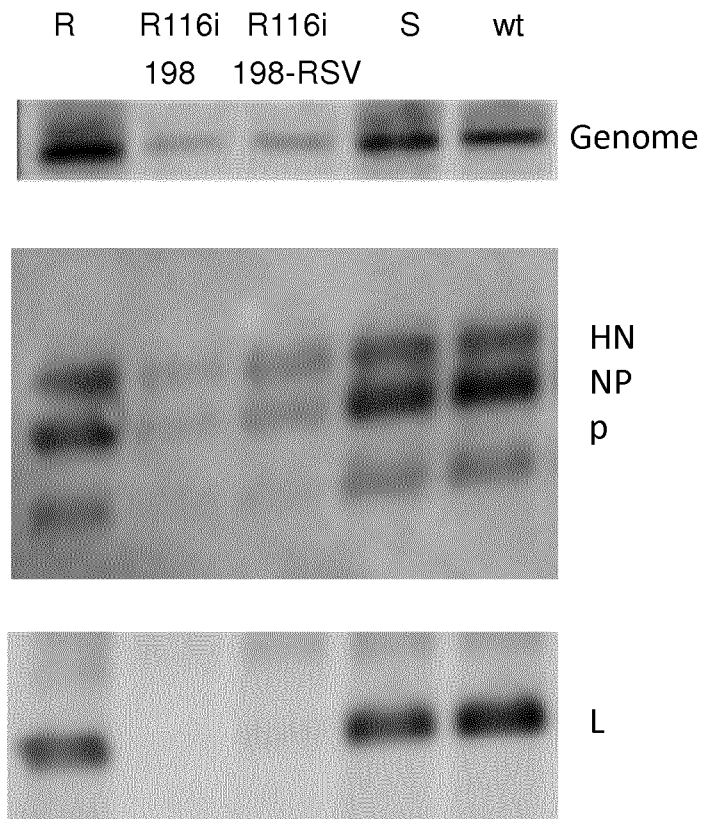
Virus Name (r73T-R116i-) ^a	Insertion at HN-L ^b	Plaque formation ^c	MDT (hrs) ^d	ICPI ^e	Relative cell killing ^f	Vero (pfu/ml) ^g	Eggs (pfu/ml) ^h
R116i	none		62	0.65	80	6.3E+06	2.0E+08
318 APMV	APMV-N 318nt		>156	0.27	80	9.9E+06	5.0E+07
198 RSV	RSV-NP 198 nt		>156	0.0375	86	6.4E+06	6.0E+07
198	Random 198 nt		>156	0	85	7.7E+06	5.0E+07
144	Random 144 nt		79	0.74	82	7.3E+06	2.4E+08
102	Random 102 nt		64	0.51	83	6.4E+06	1.1E+08
60	Random 60 nt		64	0.78	75	6.5E+06	1.3E+08
GFP	EGFP gene cassette		86	0.82	87	5.7E+06	1.5E+08

Figures 6B-E. r73T-R116i virus exhibits slower growth kinetics in DF-1 cells compared to Vero cells

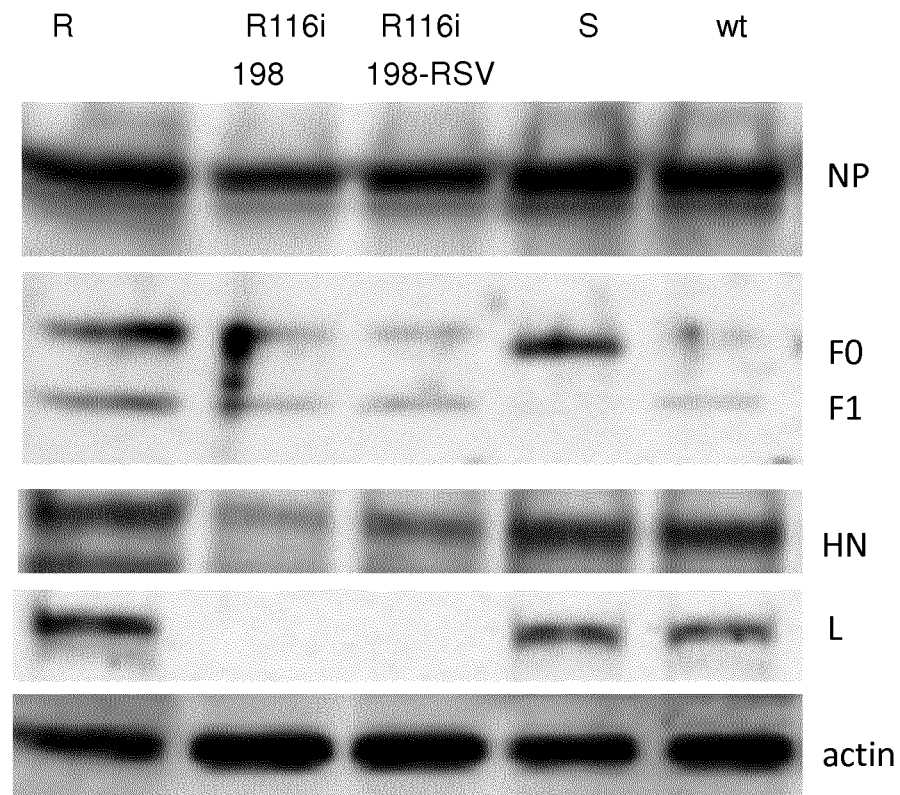


Figures 6F and G. Gene expression of r73T-R116i is reduced in DF-1 chicken fibroblast cells

F. RNA synthesis

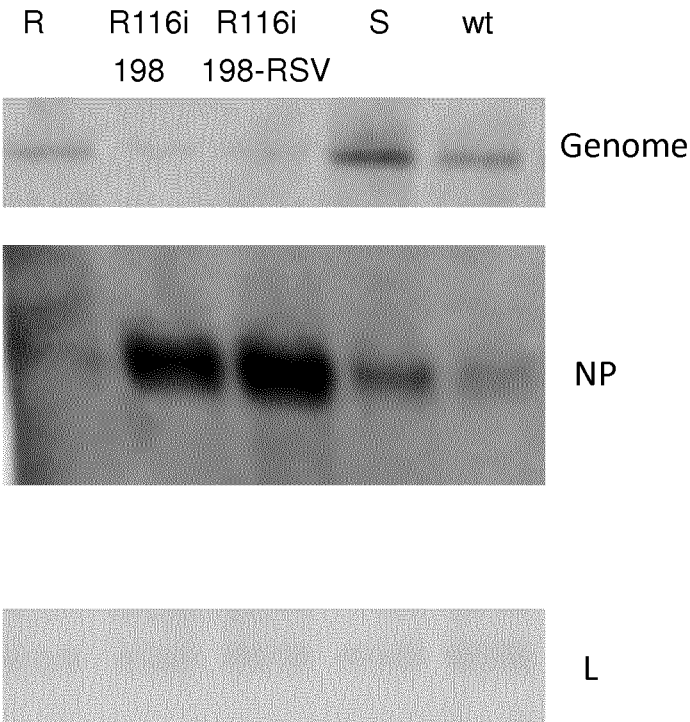


G. Protein synthesis

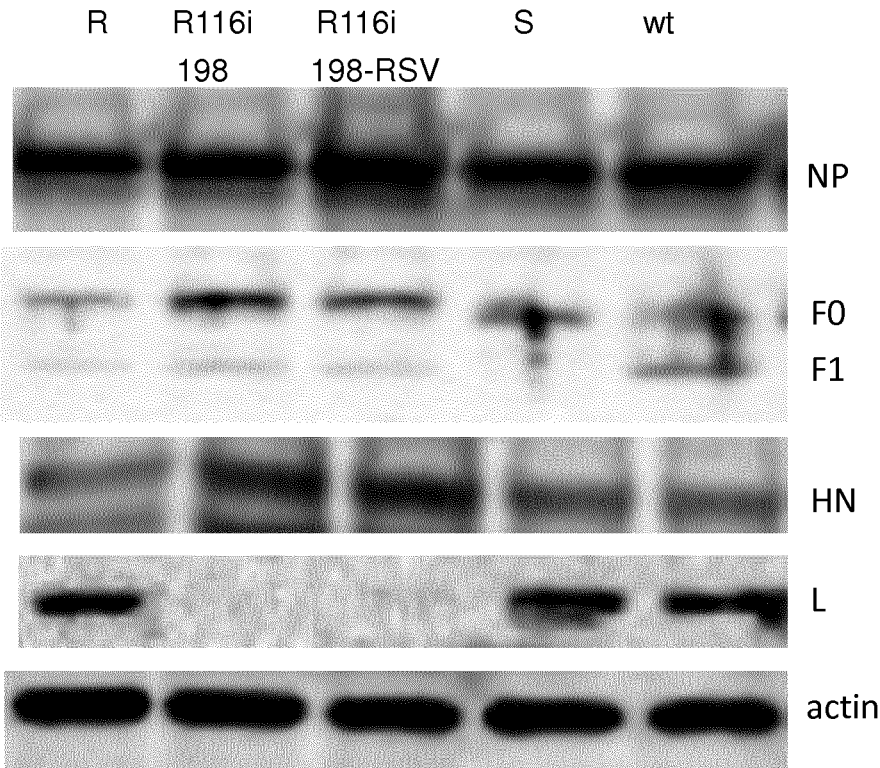


Figures 6H and I. Gene expression in the upstream of HN-L insertion is up-regulated whereas gene expression in the downstream is down-regulated in Vero cells infected by r73T-R116i virus

H. RNA synthesis

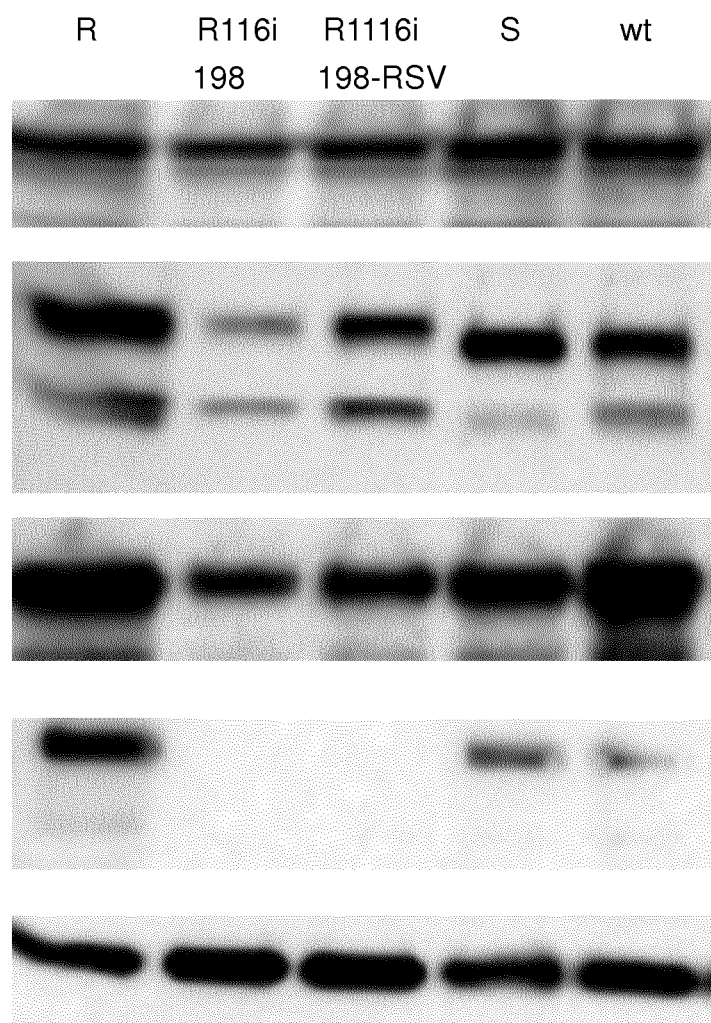


I. Protein synthesis



Figures 6J and K. Gene expression in DF-1 and Vero cells is affected by insertion at HN-L junction

J. DF-1 cell



K. Vero cell

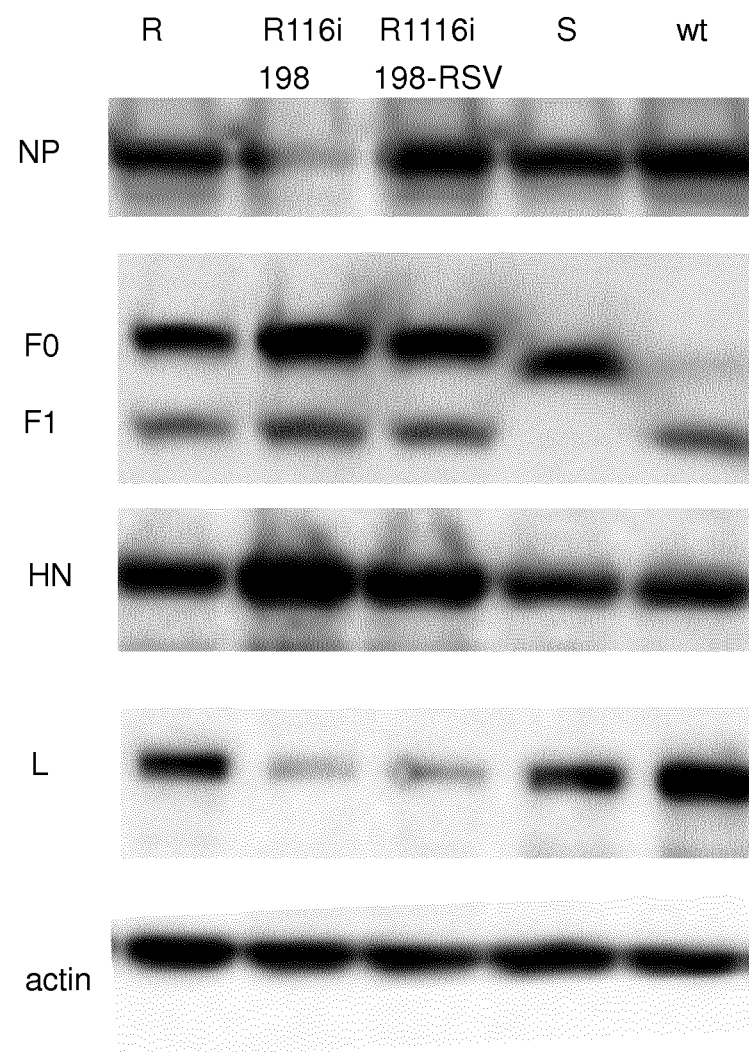


Figure 6L Expression of the upstream genes is up-regulated in r73T-R116i infected human cells

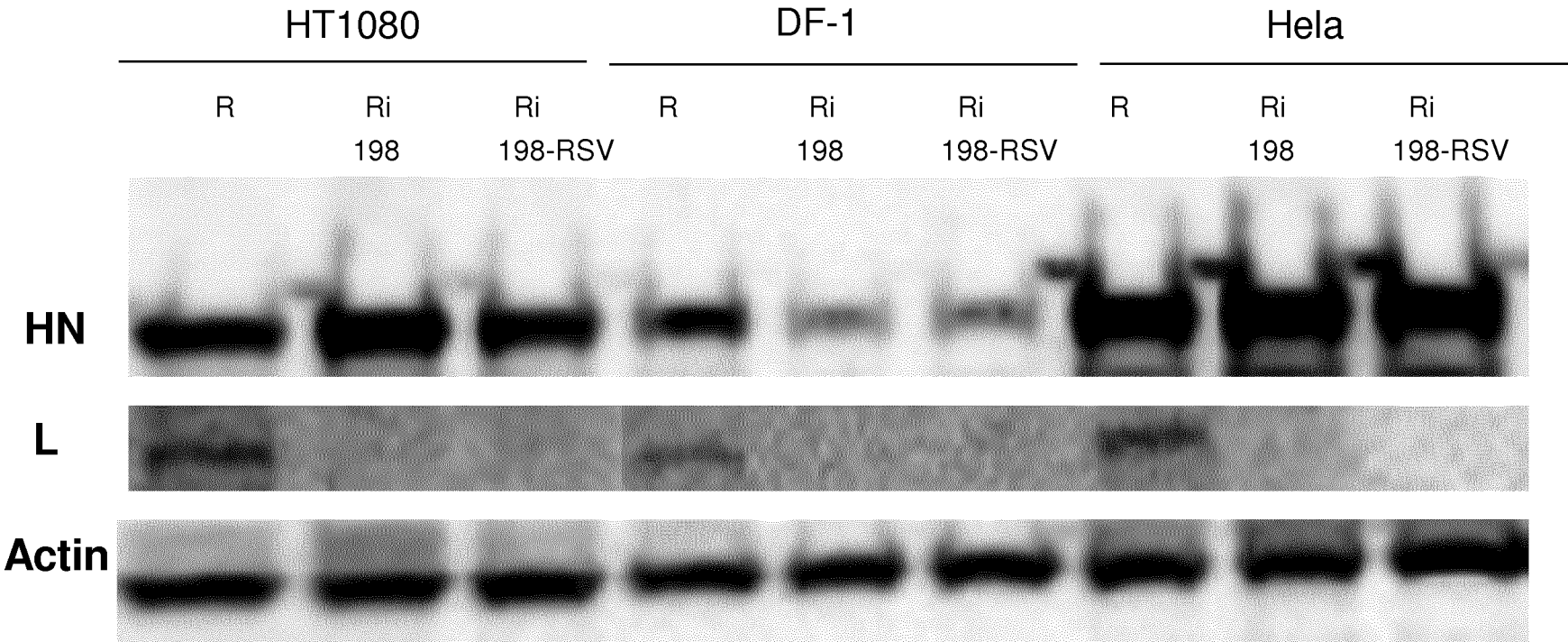


Figure 7. Growth kinetics of r73T viruses in embryonated chicken eggs

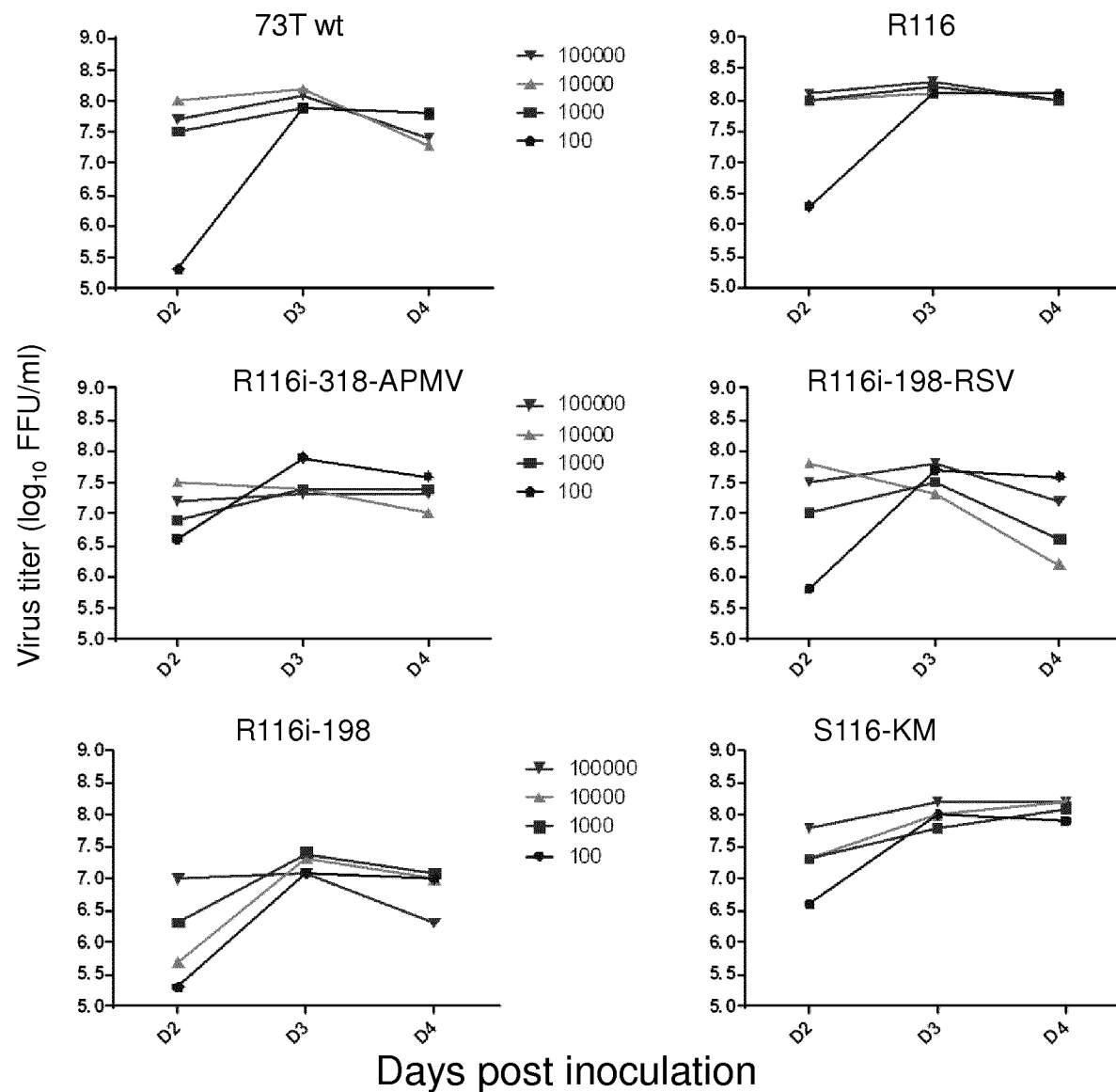


Figure 8. Growth kinetics of r73T viruses in Vero cells

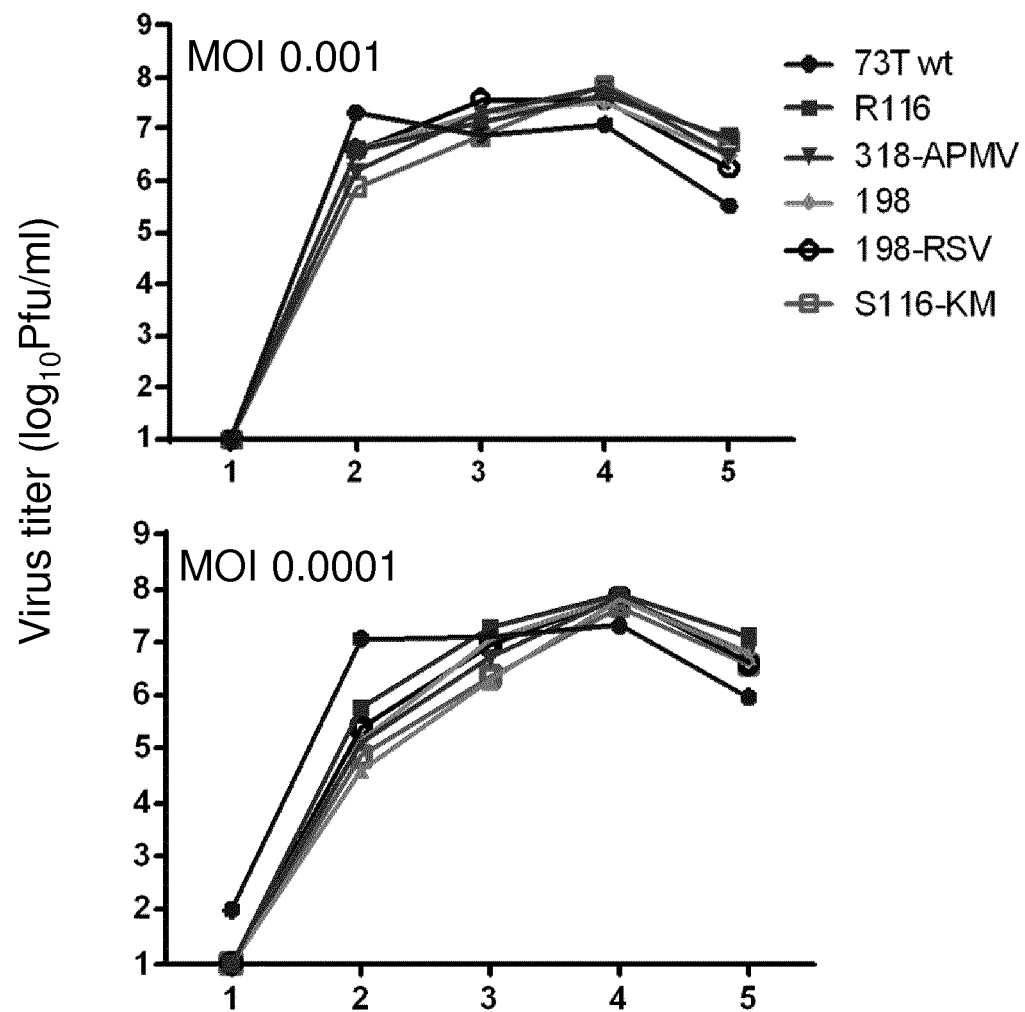


Figure 9. Selective replication and cytotoxicity of r73T virus in tumor cells

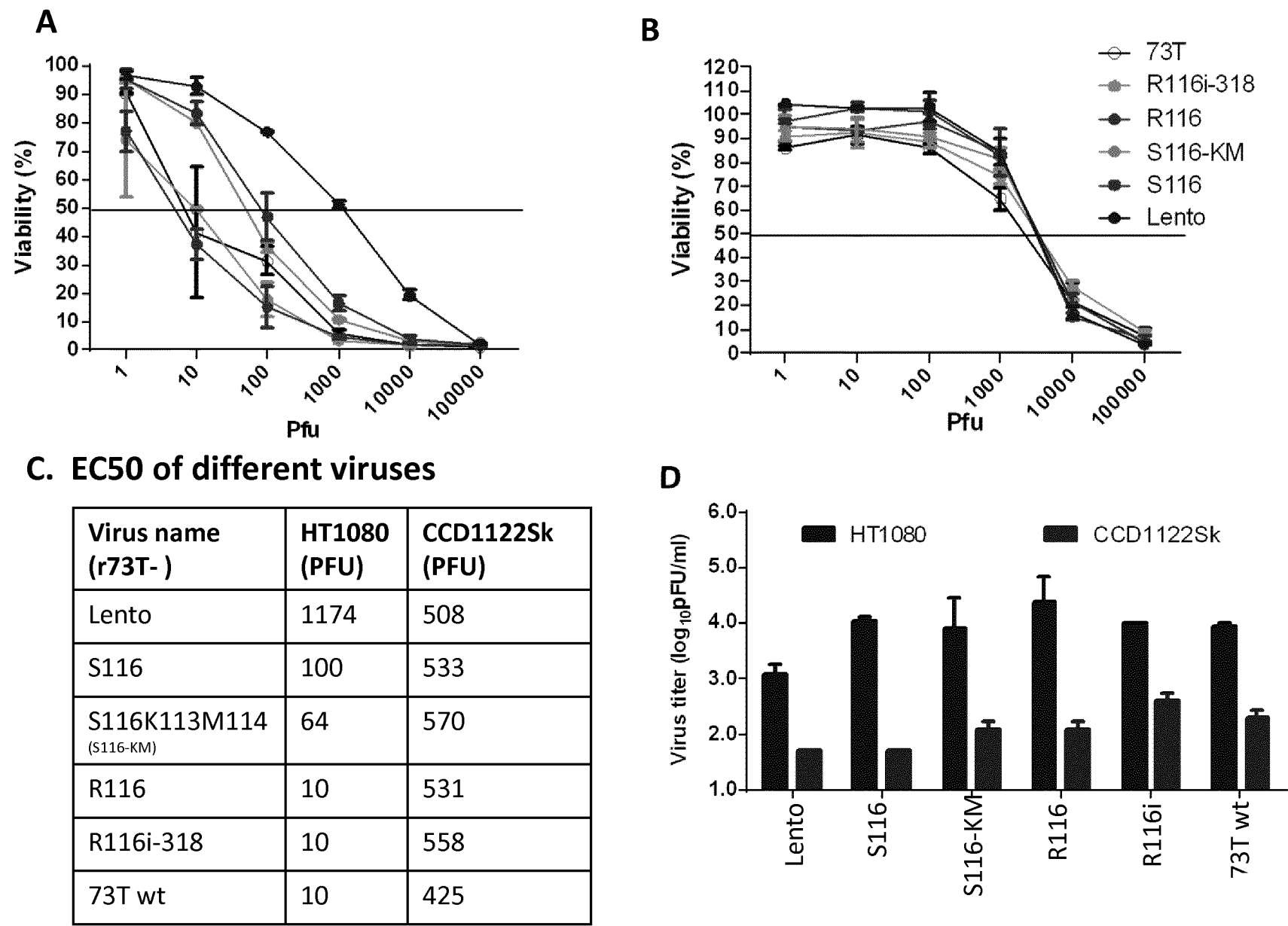
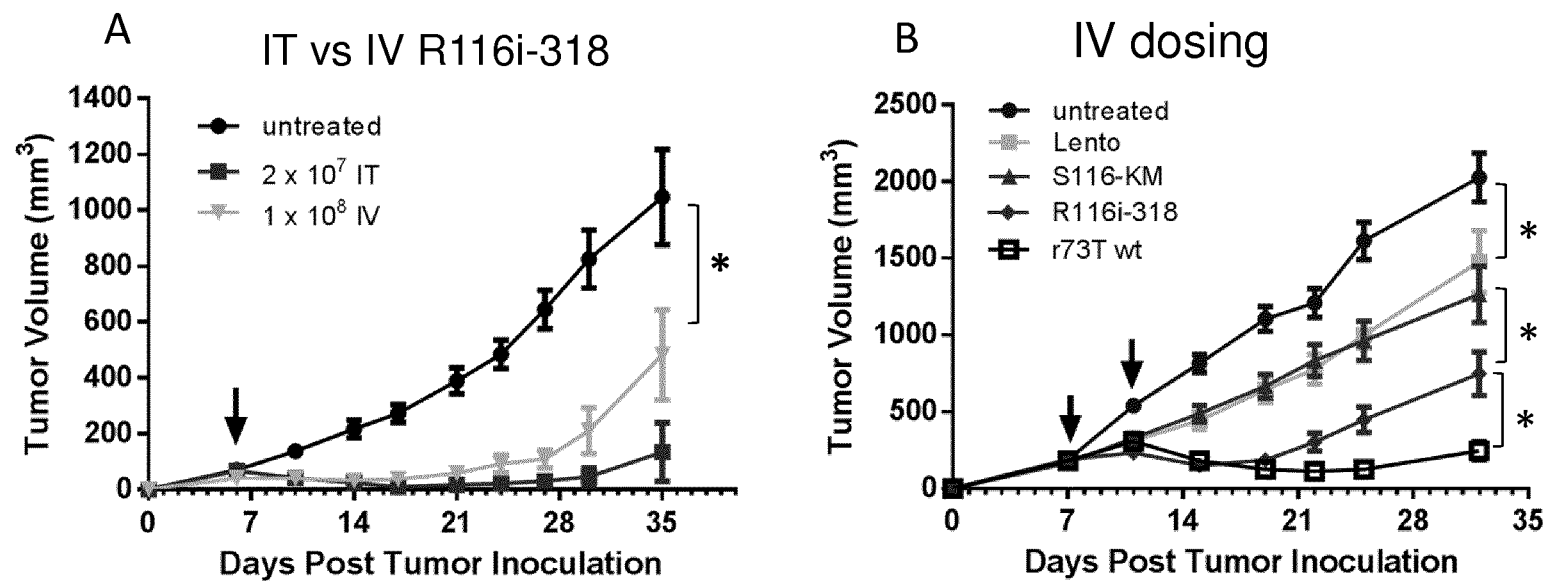


Figure 10. r73T derivatives are effective in tumor regression upon local and systemic administration



Figures 11A and B. r73T-R116i-hGM-CSF selectively replicates in Tumors following a single IV administration

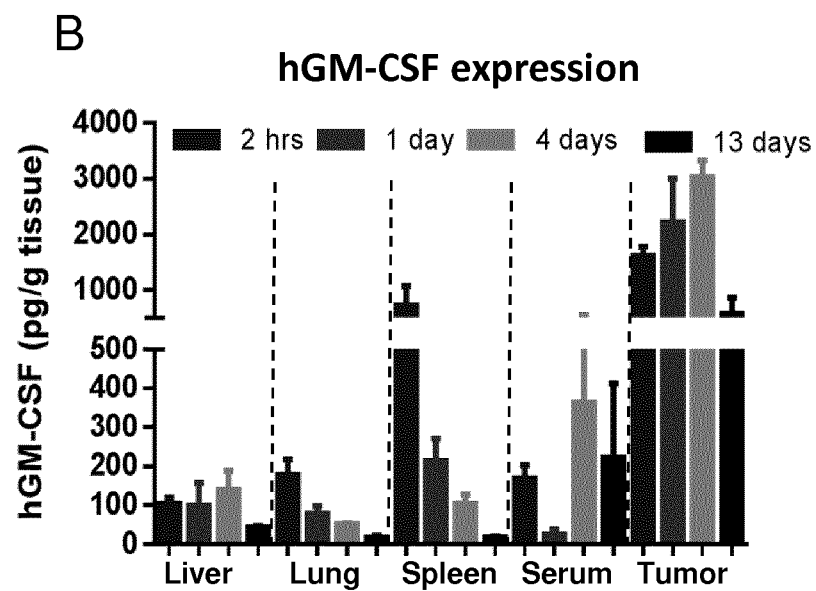
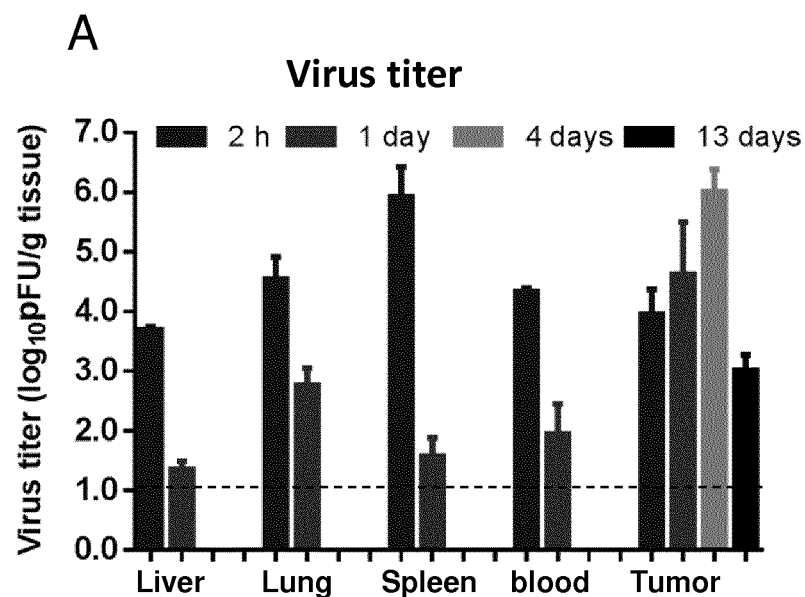


Figure 11C. Effect of mouse GM-CSF on tumor growth inhibition by IV and IT in HT1080 model

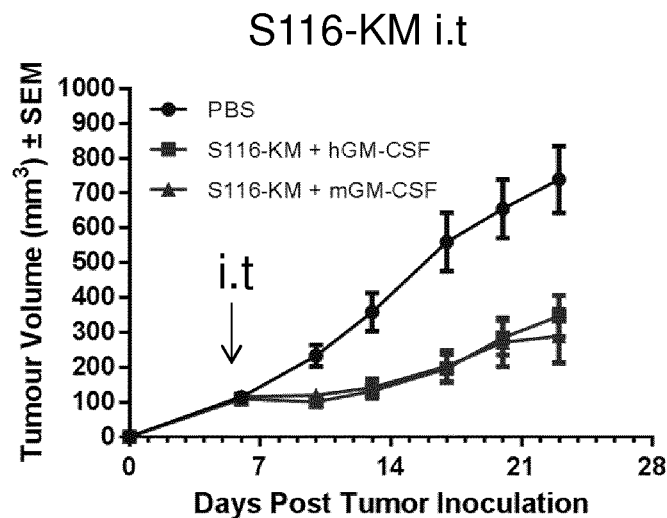
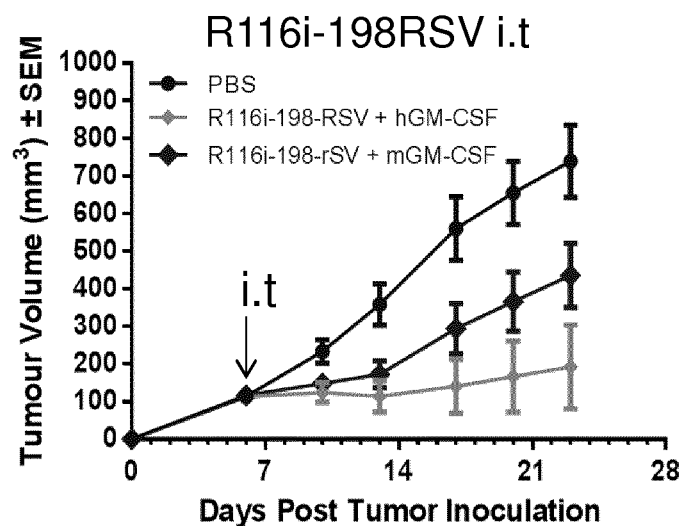


Figure 11D. Expression of mouse GM-CSF accelerated virus clearance from tumors

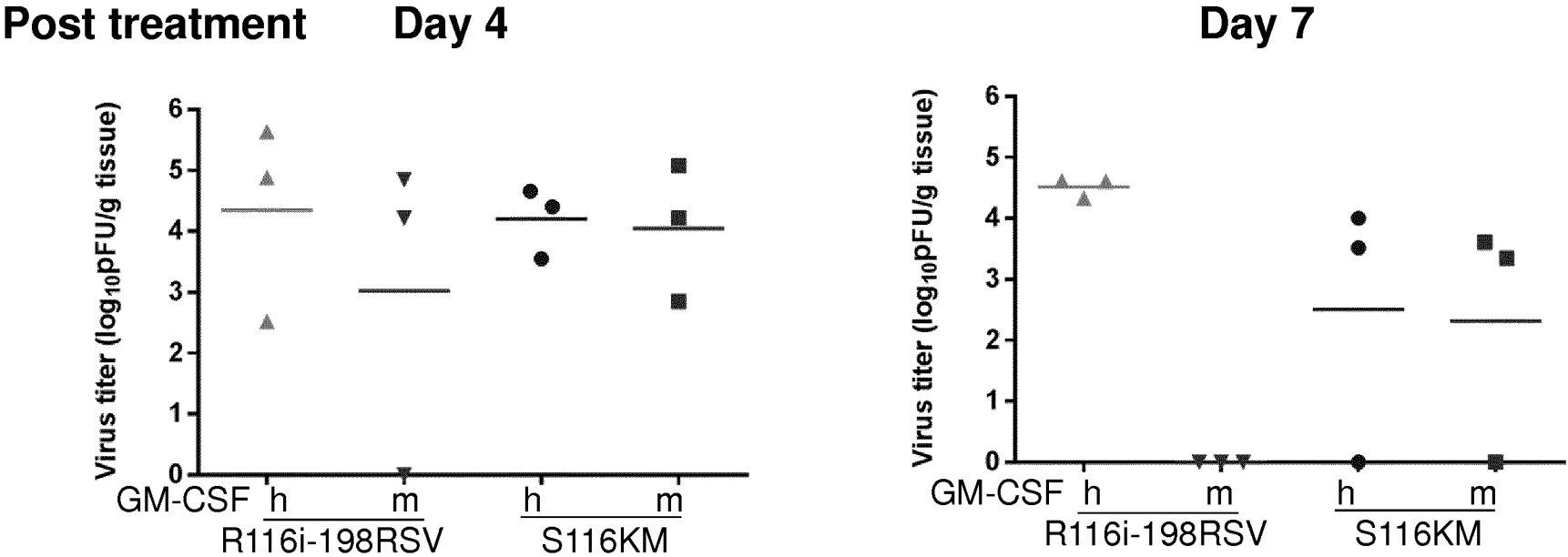


Figure 11E. Mouse GM-CSF expression induces more immune cells infiltration into tumors

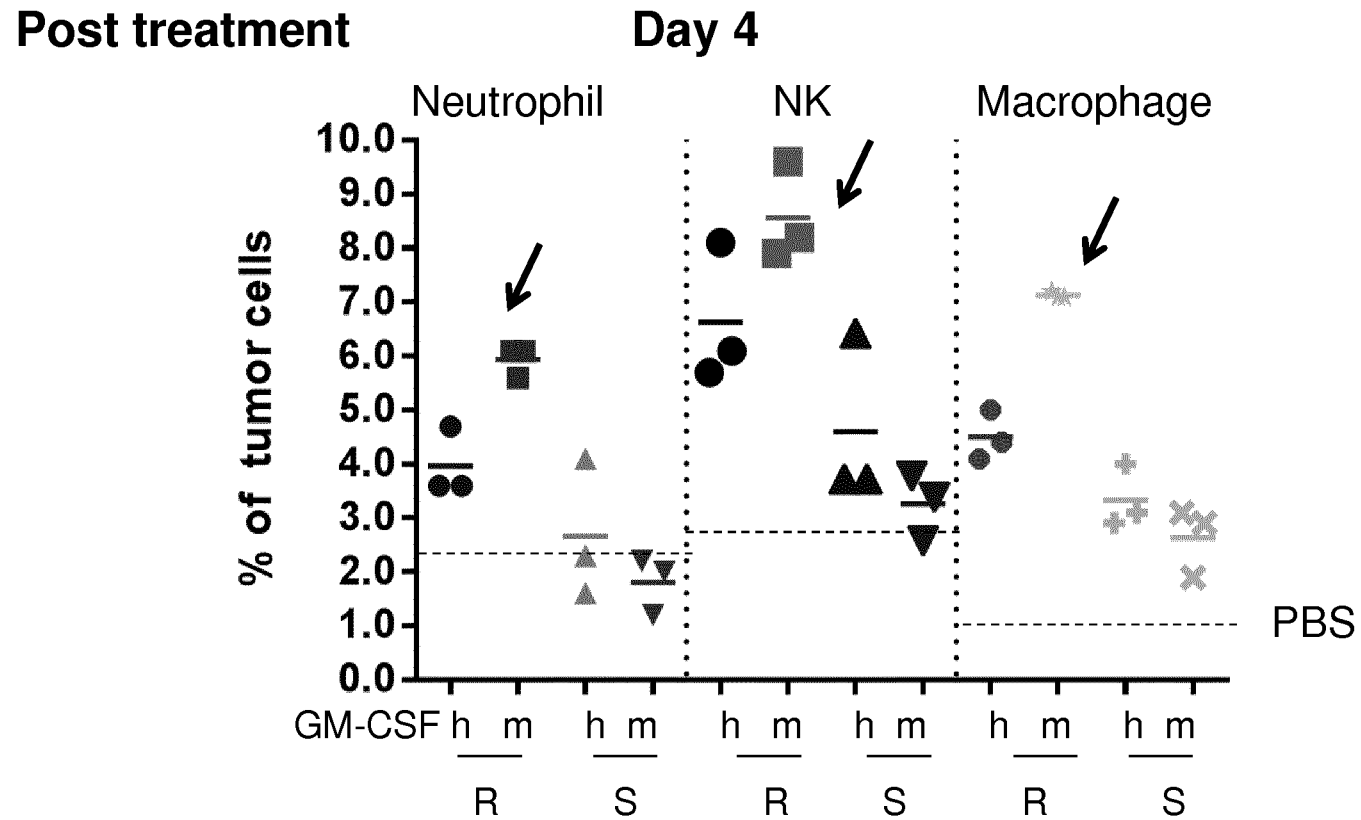


Figure 11F. rNDV treatment elicits up-regulation of cytokines and chemokines in tumor tissues

Cytokine/chemokine on day 4 post treatment	Fold of change relative to PBS group			
	R		S	
	hGM-CSF	mGM-CSF	hGM-CSF	mGM-CSF
mGM-CSF	1.3	106.7	2.4	9.9
MIG (CXCL9)	3.9	3.1	5.3	4.9
IFN- β	51	28	8	12
IFN- γ	6.9	2.7	6	2.4
IL-6	13.2	5.5	6.6	2.7
IL-10	5.7	2.8	0.5	2
MIP-1a (CCL3)	37.9	13.4	15.6	4.4
IL-12	2.9	2.5	2.4	2.3
MCP-1(CCL2)	8.4	9.5	8.9	5
TNF- α	5.2	4.7	3.6	3.7
IP-10 (CXCL10)	8.5	6.6	12.5	6.5
RANTES (CCL5)	24.5	21.2	35.9	10.1

Figure 11G. R116i-318 and -198 is comparable in tumor growth inhibition

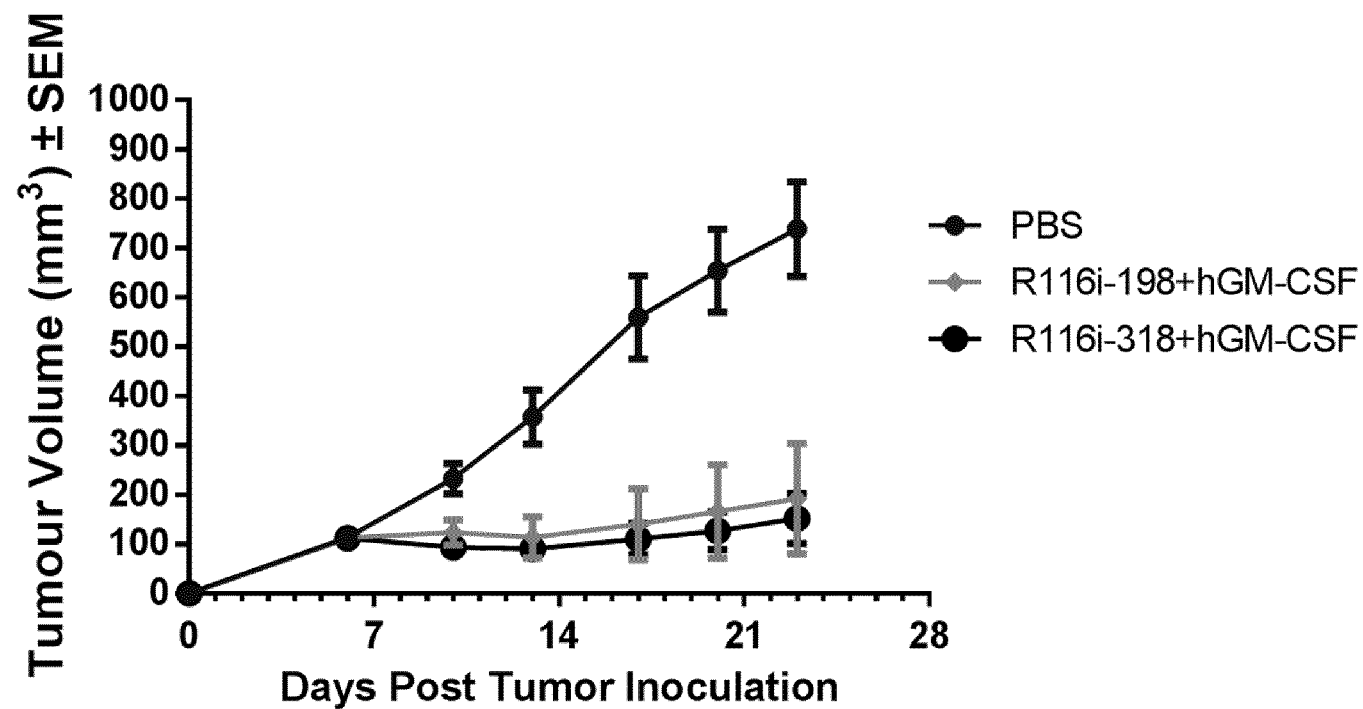


Figure 12A. Construction of antigenome cDNA of 73T containing chimeric F and/or HN gene

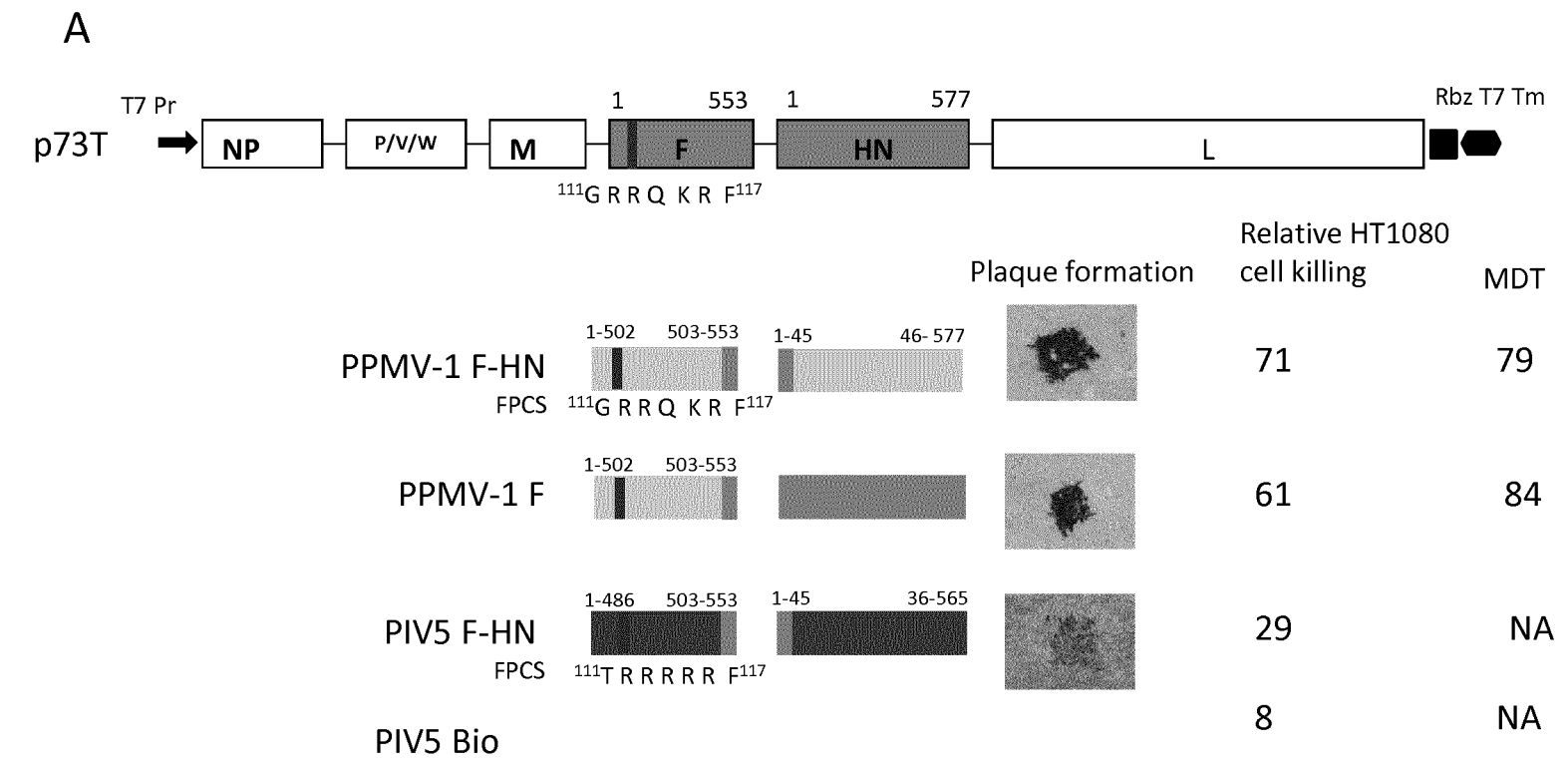


Figure 12B.

B

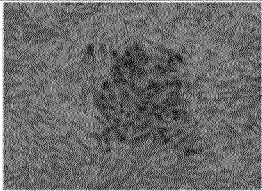
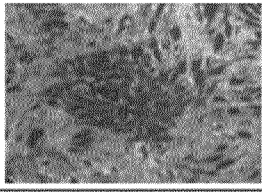
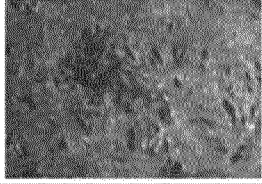
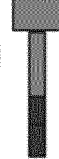
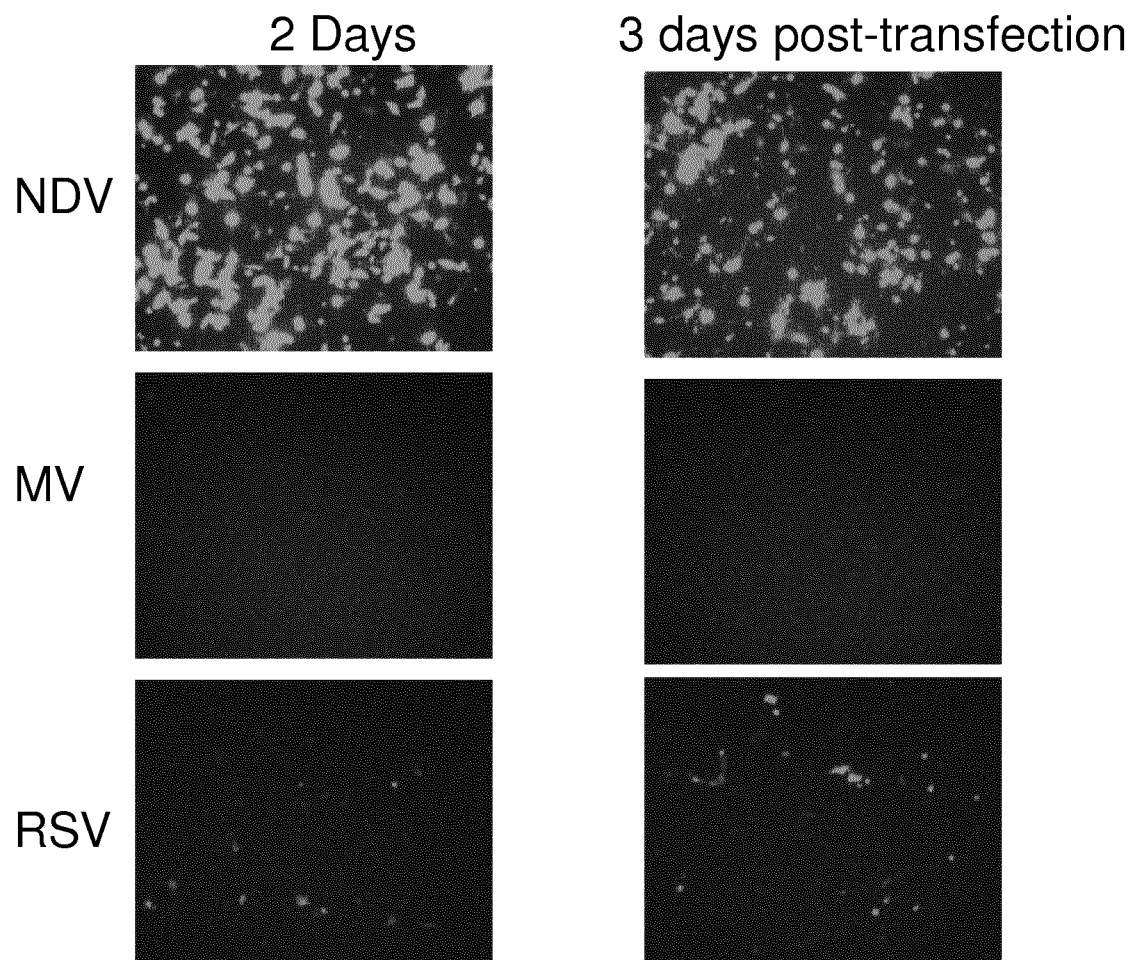
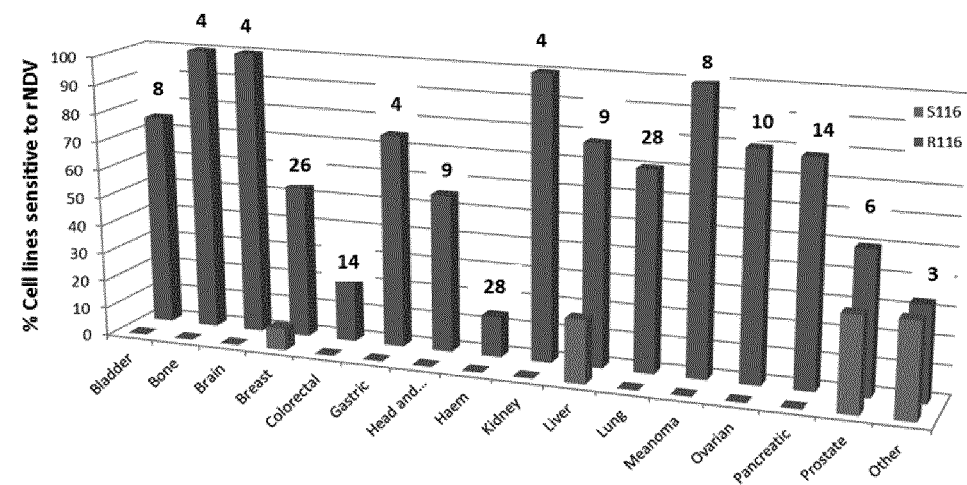
Vero w/o trypsin	Spreading in cells	F gene	HN gene	Grow in eggs (pfu/ml)	Grown in Vero w/o trypsin (pfu/ml)
	+++	PIV5 NDV 	PIV5 NDV 	No	6.0×10^6
	++++	PPMV-1 NDV 	NDV 	2.4×10^8	2.1×10^6
	+++	PPMV-1 NDV 	PPMV-1 NDV 	2.7×10^8	2.5×10^6

Figure 12C. Compare function of RNA polymerase complex by mini-genome assay



Figures 13A and B. Cancer cell line sensitivity to rNDV variants

A.



B.

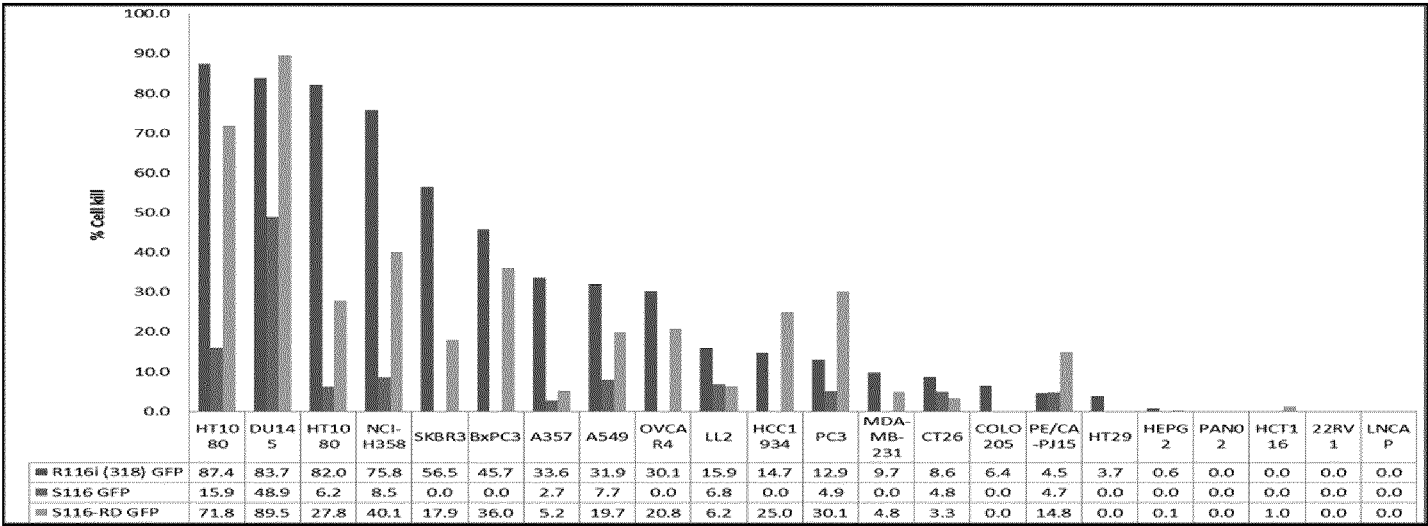


Figure 14. Permissivity of the cancer cell lines to recNDV^{GM-CSF}

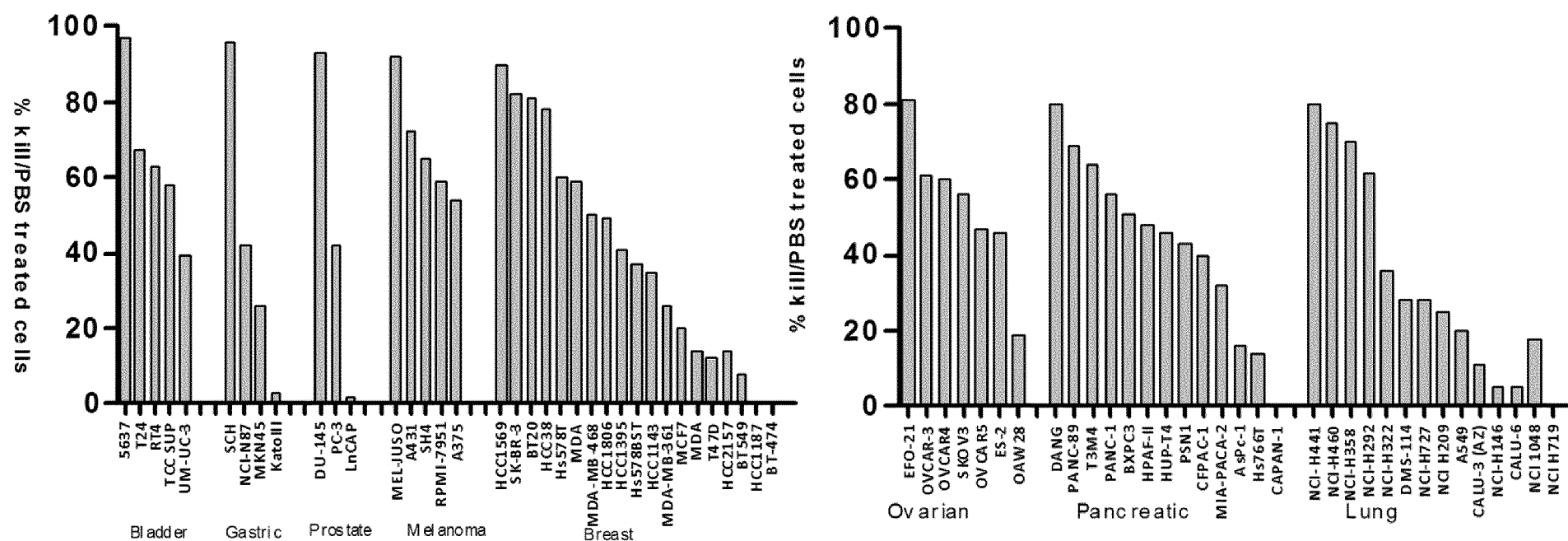
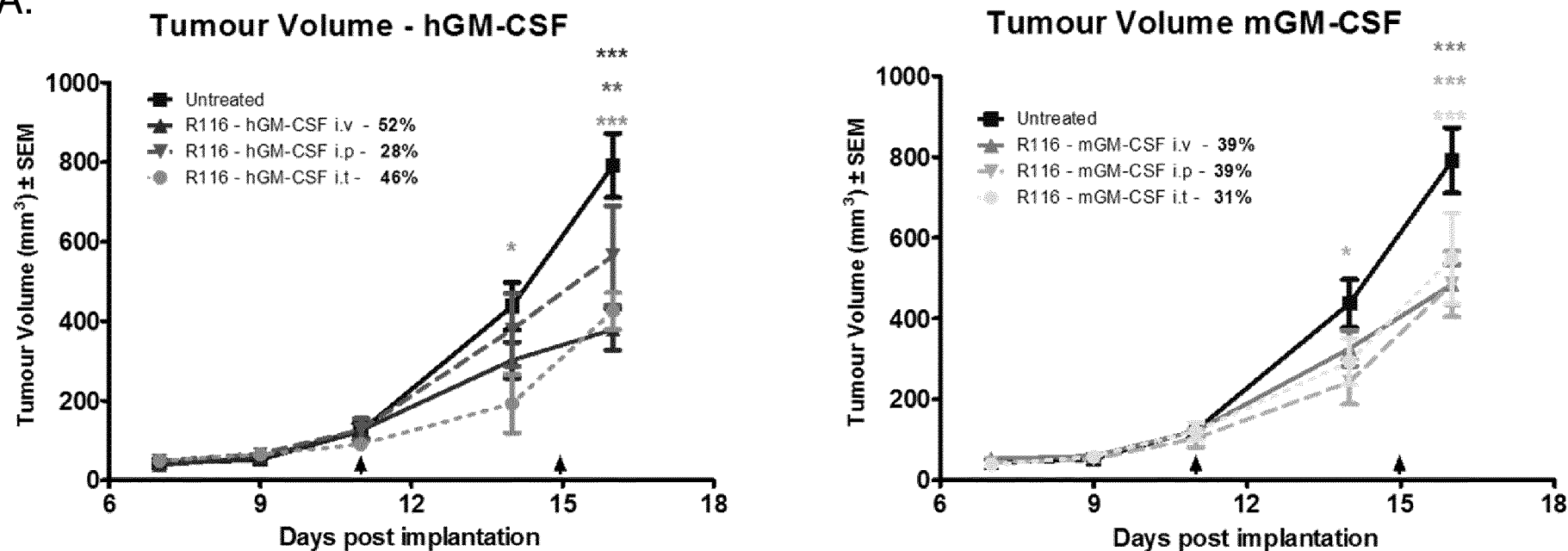


Fig legend: Cytopathic effect of rNDV variants R116i (expressing GM-CSF). Maximum % kill was determine 3 days following infection of tumour cell lines relative to non-infected control .

Figures 15A and B. Tumour growth inhibition in syngeneic mouse melanoma model (B16F10 AP3) by recNDV^{GM-CSF}

A.



B.

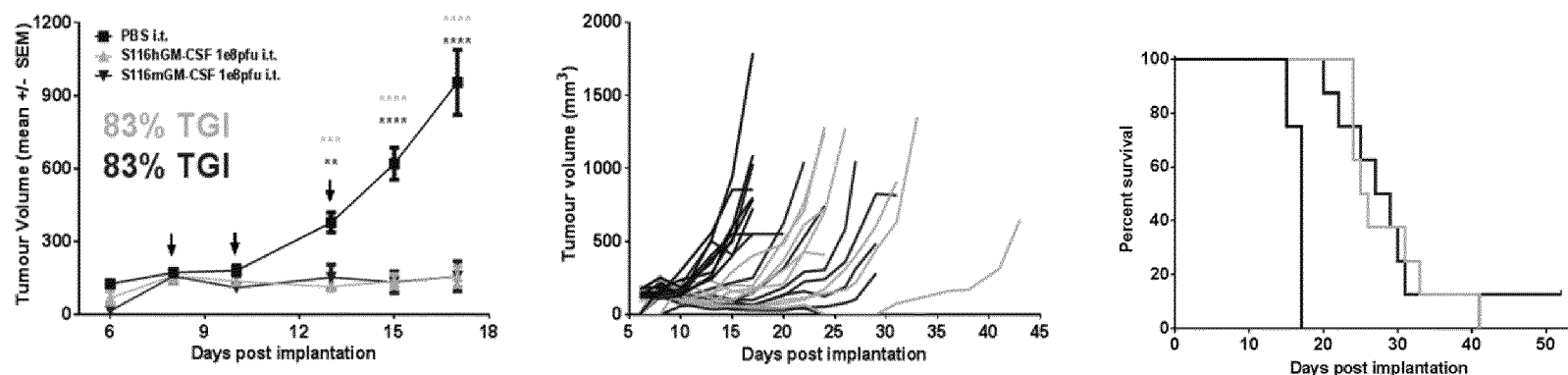
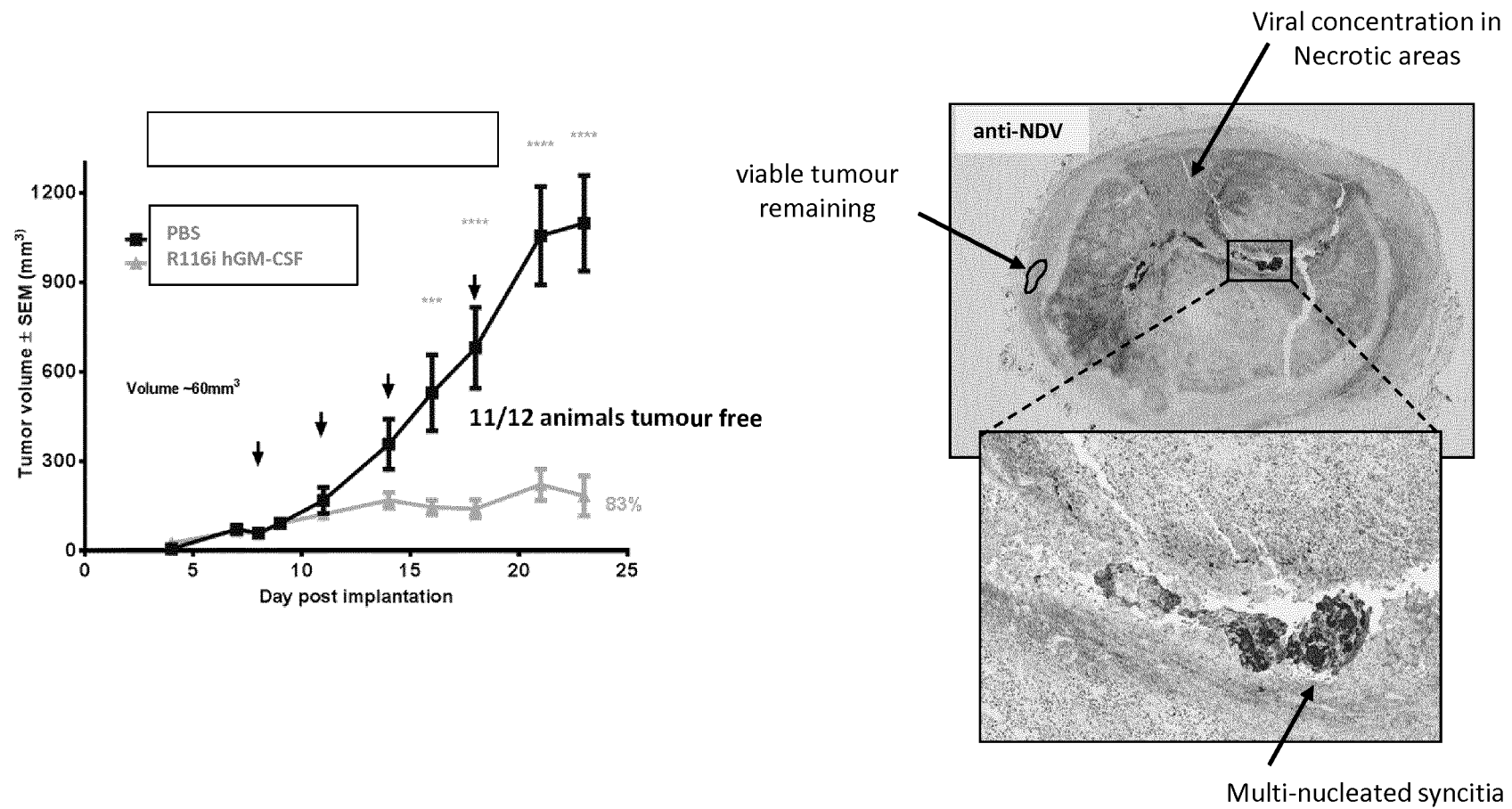


Figure 15C: NDV has potent anti-tumour activity in immune-competent mouse CT26 colorectal tumour model



Figures 15D-F Multiple dosing with rNDV R116i causes Tumour growth inhibition and drives immune cell recruitment into ovarian cancer (OVCAR4) xenograft model

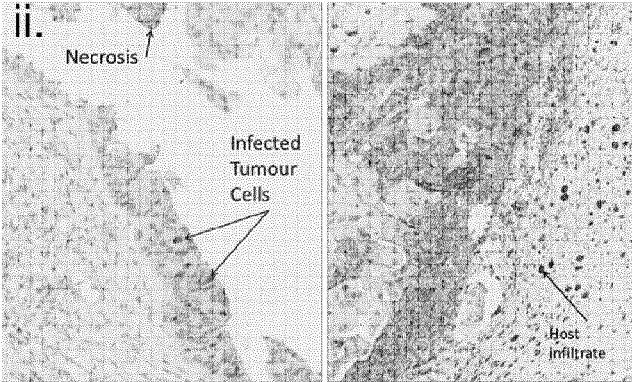
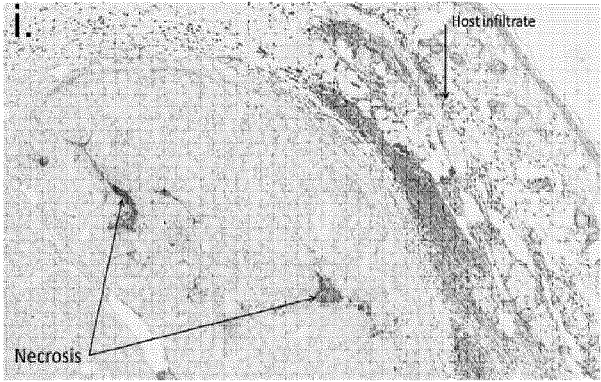
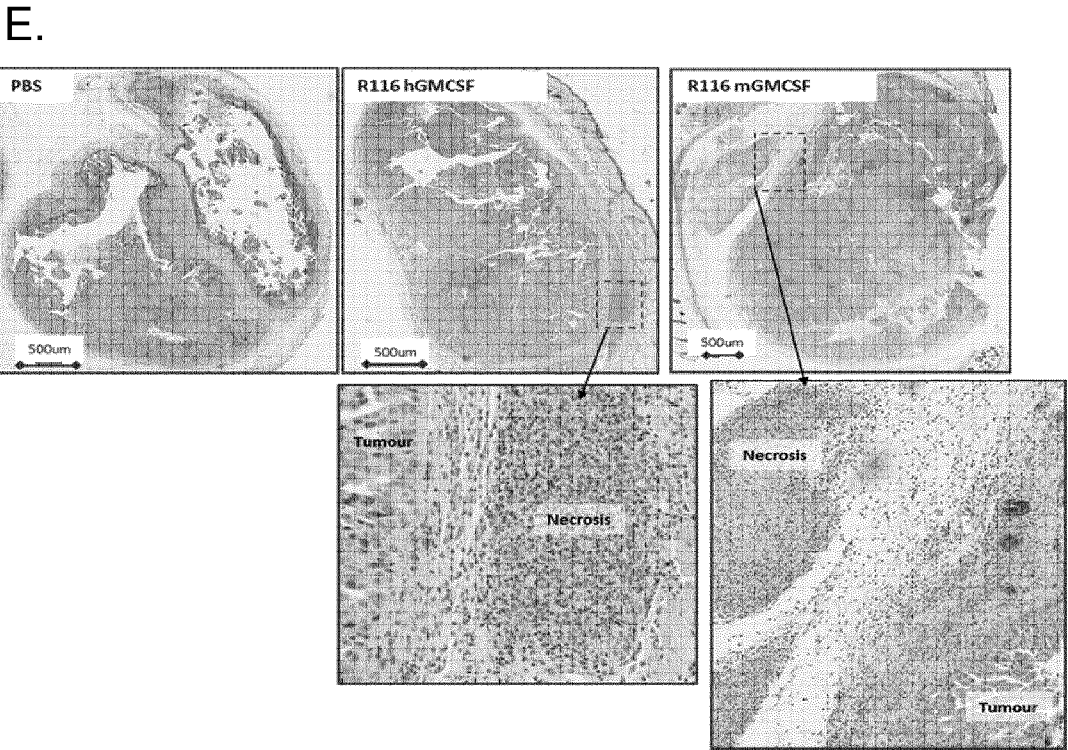
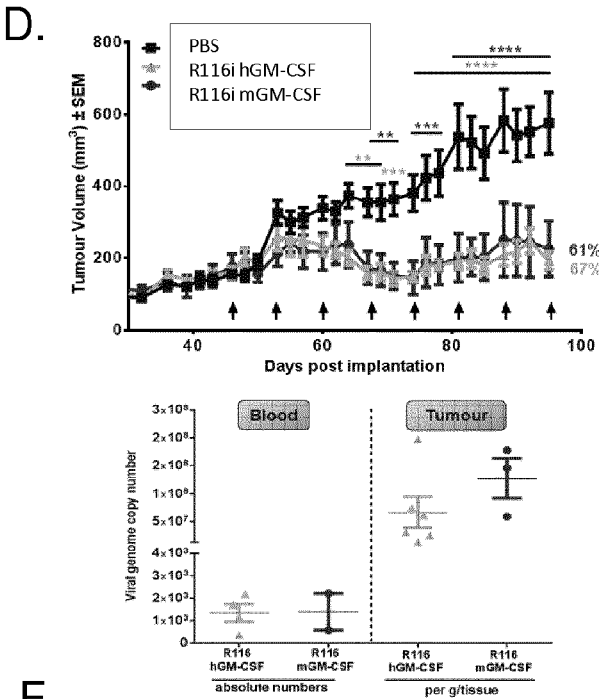
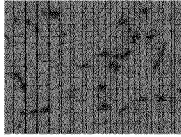
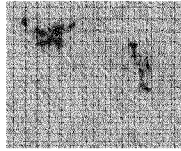
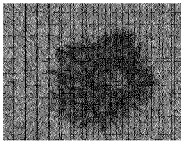
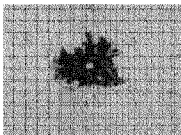
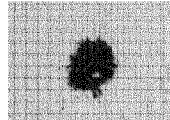
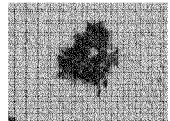


Figure 16. Summary of NDV Constructs

FPCS (R-R-Q-K-R ¹¹⁶ /F)	Plaques in Vero (no trypsin)	Transgene	HT1080 killing (%) at 72h	ICPI	comments
Lento (G-R-Q-E-R/L)		hGM-CSF	20	0.0	Replication depends on trypsin
S116 (N-R-T-K-S/F)		none	60	0.0	Do not need trypsin for intro replications
		hGM-CSF	40	0.075	Larger plaques
R116 (N-R-T-K-R/F)		none	80	tbd	Big plaques, more efficient cell killing
		hGM-CSF	80	0.65	
S116K113M114 (N-K-M-K-S/F)		hGM-CSF	48	0.00	Big plaques, intermediate efficient cell killing
R116-HN/L(318nt) (N-R-T-K-R/F)		hGM-CSF	80	0.05 - 0.27	2 loci control virus att F cleavage and intergenic stretch
R116-HN/L(198nt) (N-R-T-K-R/F)		hGM-CSF	86	0.0375	

All strains have titers of 8-9 log₁₀PFU/ml in eggs and ~7.0 log₁₀PFU/ml in Vero cells, lento cleavage site: 3 log₁₀PFU/ml in Vero.

Figure 17. NDV produced from Hela cells is less sensitive to C' mediated inactivation

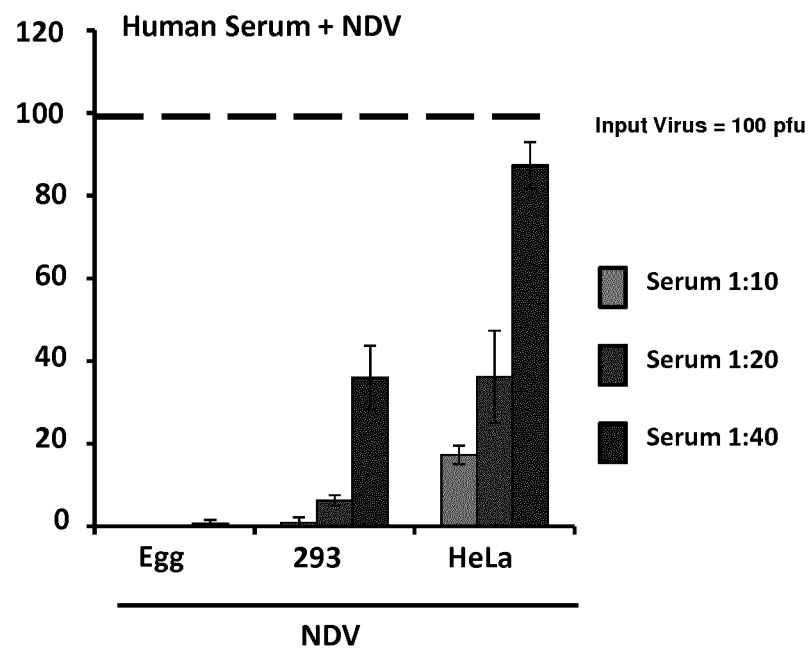


Figure 18. HeLa-3S cells express higher levels of RCA proteins than 293 cells

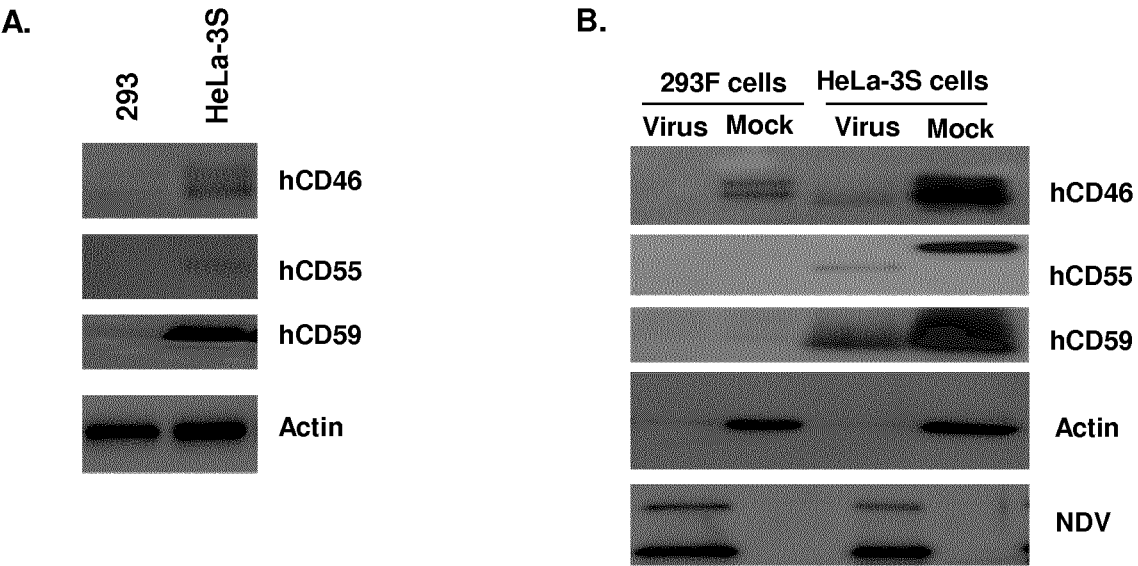


Figure 19. Expression of RCA protein by NDV

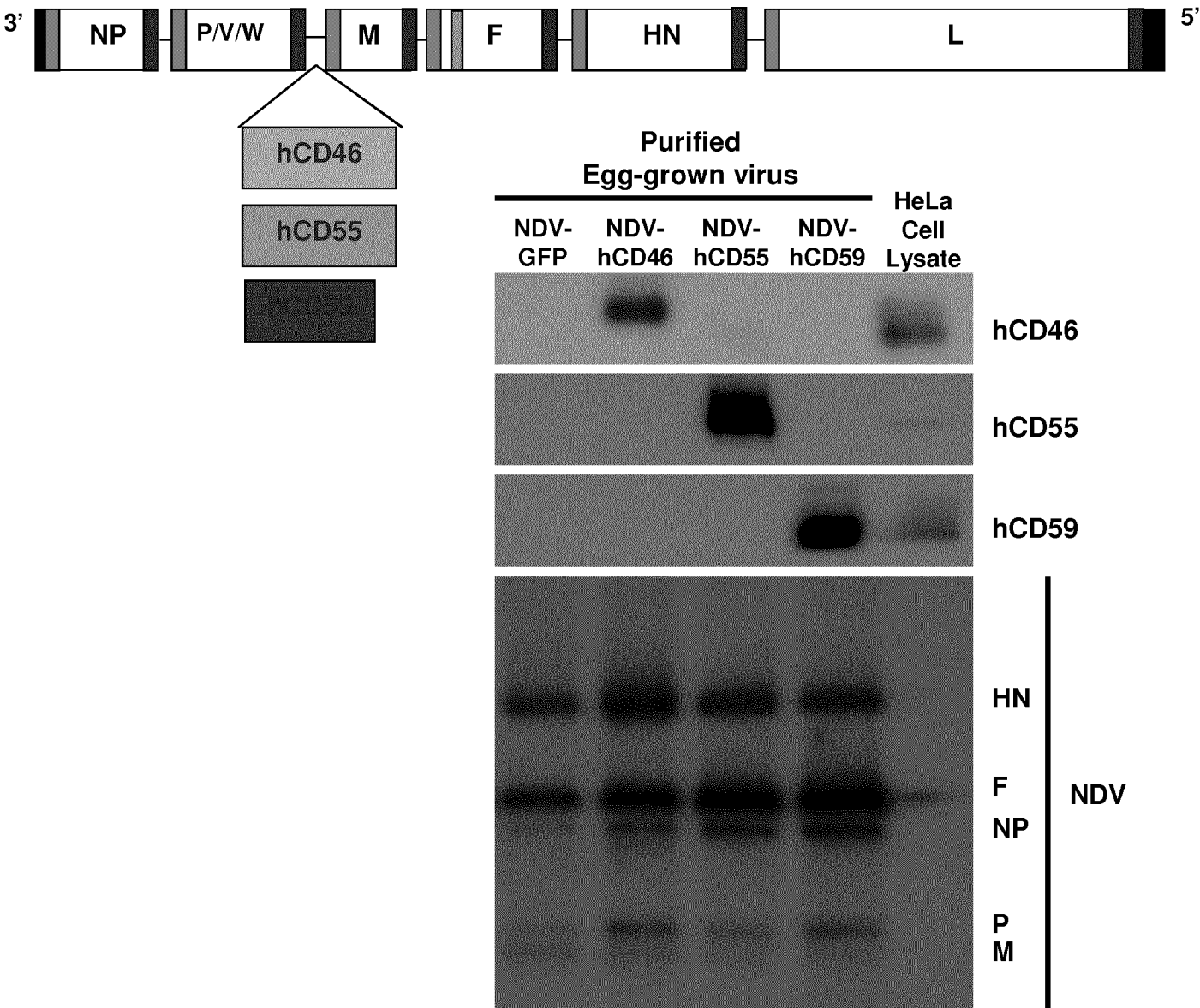


Figure 20. CD55 is a critical RCA protein for preventing C' inactivation of NDV

