frontiers

Supplementary Material

The Requirement For US28 During Cytomegalovirus Latency Is Independent Of US27 And US29 Gene Expression

Benjamin A. Krishna[1§], Amanda B. Wass[1§], Rajashri Sridharan[1], Christine M. O'Connor[1*]

1 Supplementary Data

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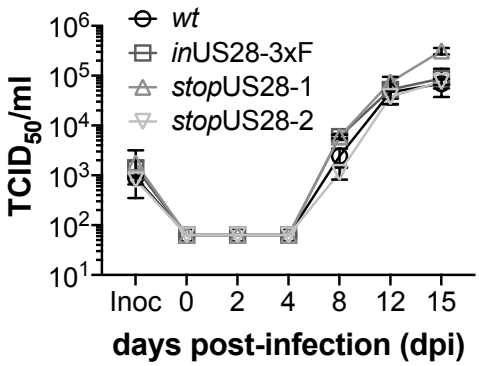
Supplementary Table 1. Oligonucleotides used in this study.

Primer Use	Primer Direction	Primer Sequence
3xF-Kan-frt insertion	forward[§]	TCTTCCGACACGCTGTCCGACGAGGTGTGTCGCGTCTCACAAATTATA CCGTTAGATTATAAAGATGATGATGATAAA
	reverse[§]	AGAGGGGCGGACACGGGGTTTGTATGAAAAGGCCGAGGTAGCGCTTT TTTAGGCCGCGGGAATTCGAAGTT
***galK* insertion**	forward[¶]	GCACCGAGGGCAGAACTGGTGCTATCATGACACCGACGACGACGACC GCGcctgttgacaattaatcatcggca
	reverse[¶]	TGAAAACACAAGGAGTCGCGTCTTCATCGTAGTCAAACTCCGTCGTGA GTTtcagcactgtcctgctcctt
***galK* reversion oligo**	top	CACCGAGGGCAGAACTGGTGCTATCATGACACCGACGACGACGACCG CGTAACTCACGACGGAGTTTGACTACGATGAAGACGCGACTCCTTGTG TTTT
	bottom	AAAACACAAGGAGTCGCGTCTTCATCGTAGTCAAACTCCGTCGTGAGT TACGCGGTCGTCGTCGTCGGTGTCATGATAGCACCAGTTCTGCCCTCG GTG
Multi-tag vGPCR virus – *galK* insertion		
UL33-c-myc	forward[¶]	ACAAAAATCCCCCATCGACTCTCACAATCGCATCATAACCTCAGCGGG GTAcctgttgacaattaatcatcggca
	reverse[¶]	AAATGGCGACGGGTTCTGGTGCTTTCTGAATAAAGTAACAGGAAAGCT CAtcagcactgtcctgctcctt
UL78-V5	forward[¶]	TGCACCGACGGCGAAAACACCGTCGCGTCCGACGCAACGGTGACGGC ATTAcctgttgacaattaatcatcggca
	reverse[¶]	GTGATTTATCTGCCACTTTTCTCCCCGCTGCCGTACAGCGCCGCCGCTC Atcagcactgtcctgctcctt
US27-3xHA	forward[¶]	TATGACAGAAAAAATGCACCTATGGAGTCCGGGGAGGAGGAATTTCT ATTGcctgttgacaattaatcatcggca

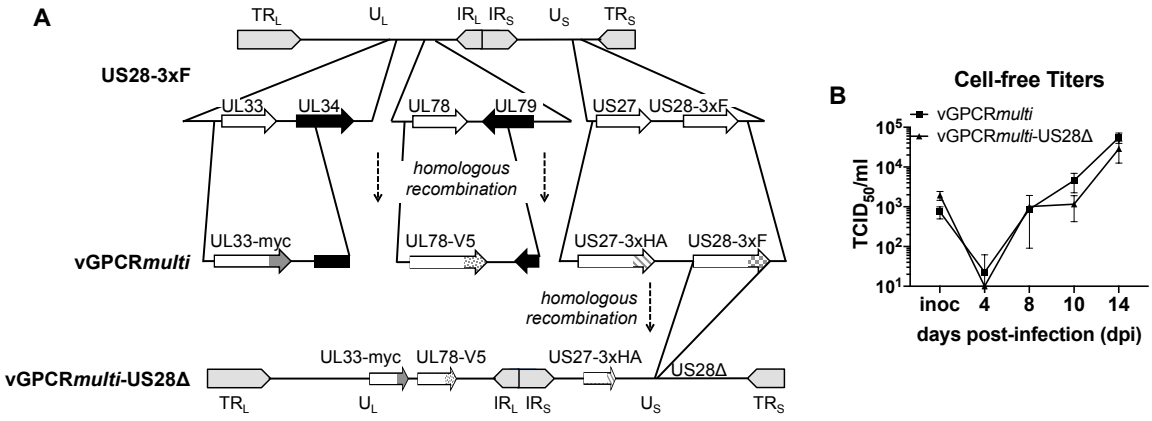
	reverse[¶]	CAATGAGCAAAAATAGATGTGCGGCGGACGCGTGAAAGAGGATCGAA TTAtcagcactgtcctgctcctt
Multi-tag vGPCR virus – *galK* reversion gBlocks		
UL33-c-myc		ACAAAAATCCCCCATCGACTCTCACAATCGCATCATAACCTCAGCGGG GTAGAACAAAAACTTATTTCTGAAGAAGATCTTTGAGCTTTCCTGTTAC TTTATTCAGAAAGCACCAGAACCCGTCGCCATTT
UL78-V5		TGCACCGACGGCGAAAACACCGTCGCGTCCGACGCAACGGTGACGGC ATTAGGTAAGCCTATCCCTAACCCTCTCCTCGGTCTCGATTCTACGTGA GCGGCGGCGCTGTACGGCAGCGGGGAGAAAAGTGGCAGATAAATCAC
US27-3xHA		TATGACAGAAAAAATGCACCTATGGAGTCCGGGGAGGAGGAATTTCT ATTGTACCCATATGACGTTCCAGACTACGCGTATCCGTACGACGTTCC GGATTACGCTTACCCTTACGACGTACCTGACTACGCTTAATTCGATCCT CTTTCACGCGTCCGCCGCACATCTATTTTTGCTCATTG
Multi-tag vGPCR virus – *galK* reversion gBlock amplifying primers		
UL33-c-myc	forward	ACAAAAATCCCCCATCGACTC
	reverse	AAATGGCGACGGGTTCTGGTG
UL78-V5	forward	TGCACCGACGGCGAAAACACC
	reverse	GTGATTTATCTGCCACTTTTC
US27-3xHA	forward	TATGACAGAAAAAATGCACCT
	reverse	CAATGAGCAAAAATAGATGTG
RTqPCR		
US27	forward	CCGTATGGTGCGGTTTATCATTA
	reverse	CTAAAAATAGCGCCAGGTTGAAAGG
US28	forward	CCAGAATCGTTGCGGTGTCTCAGT
	reverse	CGTGTCCACAAACAGCGTCAGGT
US29	forward	CGACGAGACAACAATGAC

	reverse	AATTGACGGTCCACTGAG
UL123	forward	GCCTTCCCTAAGACCACCAAT
	reverse	ATTTTCTGGGCATAAGCCATAATC
UL99	forward	GTGTCCCATTCCCGACTCG
	reverse	TTCACAACGTCCACCCACC
GAPDH	forward	ACCCACTCCTCCACCTTTGAC
	reverse	CTGTTGCTGTAGCCAAATTCGT

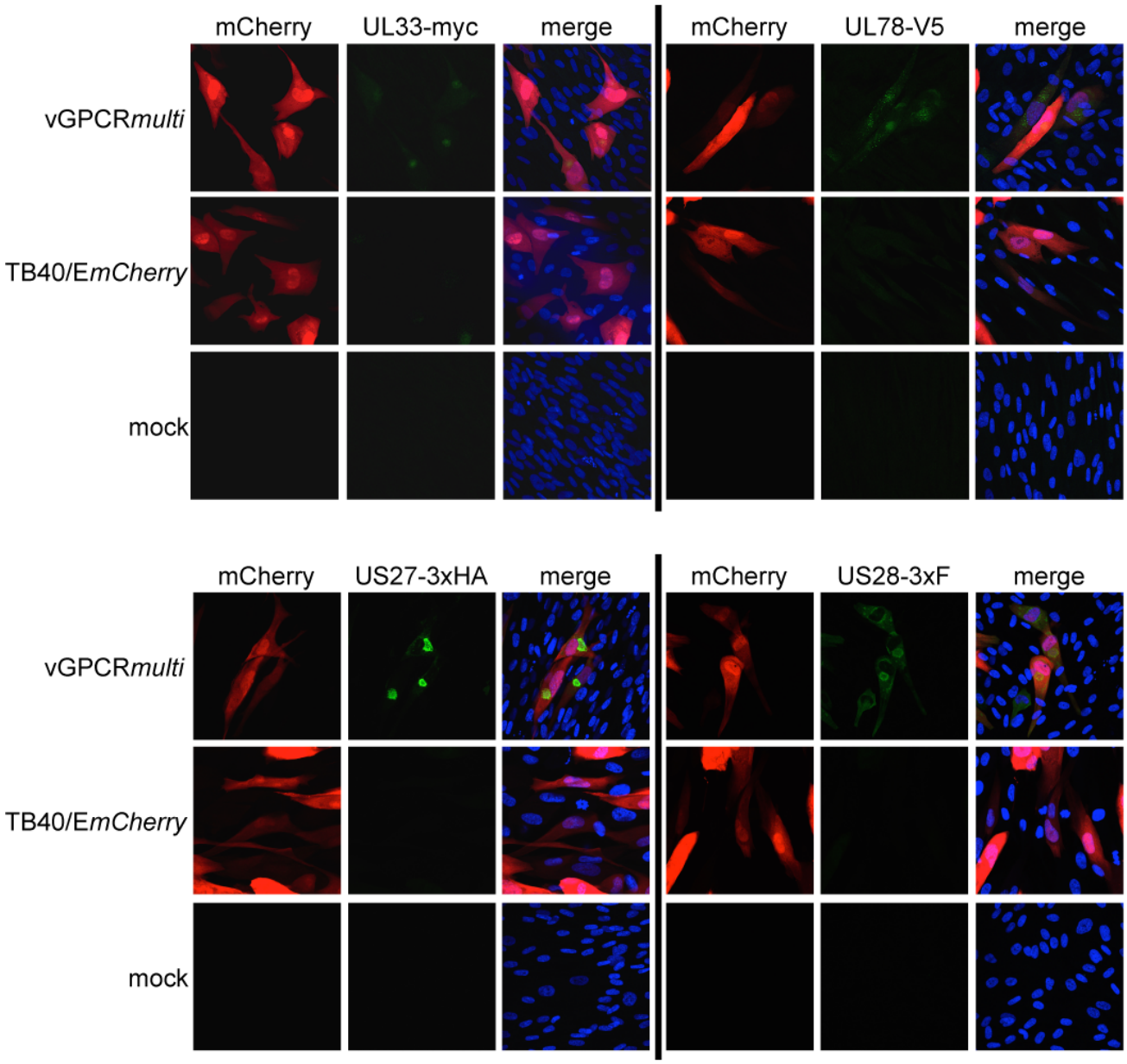
[§] Underlined sequences are complementary to the pKan-frt template.
[¶] Lowercase sequences are complementary to the pGalK template.



Supplementary Figure 1. US28 recombinant viruses display wild type growth kinetics in lytically-infected primary fibroblasts. MRC-5 cells were infected as indicated (moi = 0.01). Supernatants from each culture were collected at the indicated days post-infection (dpi). Infectious virus was quantified by $TCID_{50}$ on naïve fibroblasts. Inoc, inoculum. All samples were analyzed in triplicate. Error bars indicate standard deviation, and statistical significance was calculated using two-way ANOVA analyses followed by Tukey's post-hoc analyses. Values were not statistically significant.



Supplementary Figure 2. TB40/E*mCherry*-vGPCR*multi* and- vGPCR*multi*-US28Δ replicate to wild type titers in lytically infected fibroblasts. (A) TB40/E*mCherry*-US28-3xF (Miller et al., 2012) was used to generate TB40/E*mCherry*-vGPCR*multi* (vGPCR*multi*), containing epitope tags on each additional vGPCR, as depicted. vGPCR*multi* was subsequently used to generate vGPCR*multi*-US28Δ, which lacks the entire US28 ORF, inclusive of the triple FLAG epitope tag. **(B)** Multi-step growth analyses of the viral recombinants in **(A)** was evaluated in lytically infected NuFF-1 fibroblasts (moi = 0.01). At the indicated times post-infection, infectious supernatants were collected, and titers were determined using $TCID_{50}$ assay on naïve NuFF-1 fibroblasts in triplicate. Inoc, inoculum. Error bars indicate standard deviation, and statistical significance was calculated using two-way ANOVA with Sidak's post-hoc analysis. Values were not statistically significant.



Supplementary Figure 3. TB40/E*mCherry*-vGPCR*multi* expresses each vGPCR following lytic infection. NuFF-1 fibroblasts were mock-infected or lytically infected (moi = 0.5) as shown and harvested 96 hpi for IFA. The appropriate antibodies were used to detect the indicated epitope tag (see Materials and Methods for specific antibody details): UL33 (anti-myc), UL78 (anti-V5), US27 (anti-HA), US28 (anti-FLAG), each shown in green. mCherry (red) is a marker of infection, and nuclei were visualized with DAPI (blue). Images were acquired with a 40x objective with 1.5x magnification. Representative images are shown (n=3).