Supplementary Figures

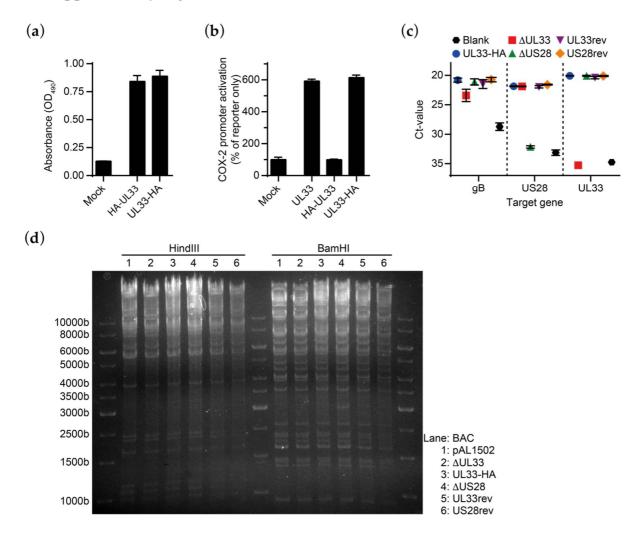


Figure S1. Validation of recombineering strategy and genomic integrity of HCMV Merlin BAC variants. Functionality check of N- or C-terminally HA-tagged UL33 receptors. HEK293T cells were transfected with receptor DNA and/or COX-2 promoter-driven reporter gene. The next day, receptor expression (a) and receptor signaling (b) were determined. Quality control of HCMV Merlin BAC derivatives as assessed by qRT-PCR of US28 and UL33 genes (c) and HindIII and BamHI endonuclease digestion pattern analysis (d).

<u>Strain</u>	UL33 receptor amino acid sequence	Residue
Merlin Towne Toledo TB40/E	MDTIIHNTTNRSTDTPHVNITCNITEPLSAIRTTEAV <mark>I</mark> NTFIIFVGGPLNAIVL <mark>I</mark> TQLLT MDTIIHNTTNRSTSTPHVNSTCNMTETLSAIRTTEAVINTEIIFVGGPLNAIVL <mark>V</mark> TQLLT MDTIIHNST-RNTTTPHINDTCNMTGPLFAIRTTEAVLNTLIIFVGGPLNAIVLVTQLLT MDTIIHNST-RNNTPPHINDTCNMTGPLFAIRTTEAVLNTFIIFVGGPLNAIVL <mark>I</mark> TQLLT	60 60 59 59
AD169 FIX/PH/TR	MDTIIHN <mark>ST-RNNTP</mark> PH <mark>IND</mark> TCNMTGPLFAIRTTEAVLNTFIIFVGGPLNAIVL <mark>I</mark> TQLLT MDTIIHN <mark>S</mark> T-RNNTPPHINDTCNMTGPLFAIRTTEAVLNTFIIFVGGPLNAIVL <mark>I</mark> TQLLT ******* * **:* ***:* * ************	59 59
Merlin	NRVLGYSTPTIYMTNLYSTNFLTLTVLPFIVLSNQWLLPA <mark>S</mark> V <mark>A</mark> SCKFLSVIYYSSCTVGF	120
Towne	NRVLG STPTIYMTNLYSTNFLTLTVLPFIVLSNQWLLPASVTSCKFLSVIYYSSCTVGF	120
Toledo TB40/E	NRVLGYSTPTIYMTNLYSTNFLTLTVLPFIVLSNQWLLPA <mark>GVA</mark> SCKFLSVIYYSSCTVGF NRVLGYSTPTIYMTNLYSTNFLTLTVLPFIVLSNQWLLPA <mark>GVA</mark> SCKFLSVIYYSSCTVGF	119 119
AD169	NRVLGYSTPTIYMTNLYSTNFLTLTVLPFIVLSNOWLLPAGVASCKFLSVIYYSSCTVGF	119
FIX/PH/TR	NRVLGYSTPTIYMTNLYSTNFLTLTVLPFIVLSNQWLLPAGVASCKFLSVIYYSSCTVGF ************************************	119
Merlin	ATVALIAADRYRVLHKR <mark>T</mark> YARQSYRSTY <mark>I</mark> IL <mark>L</mark> LTW <mark>F</mark> AGLIFS <mark>M</mark> PAAVYTTVV <mark>I</mark> H <mark>NGTNG-</mark>	179
Towne	ATVALIAADRYRVLHKR <mark>T</mark> YARQSYRSTY <mark>I</mark> IL <mark>L</mark> LTW <mark>F</mark> AGLIFS <mark>M</mark> PAAVYTTVV <mark>IHNGTDE-</mark>	179
Toledo	ATVALIAADRYRVLHKR <mark>I</mark> YARQSYRSTY <mark>T</mark> IL <mark>I</mark> LTW <mark>L</mark> AGLIFS <mark>M</mark> PAAVYTTVV <mark>MHHEANDT</mark>	179
TB40/E	ATVALIAADRYRVLHKR <mark>T</mark> YARQSYRSTY <mark>M</mark> IL <mark>L</mark> LTW <mark>L</mark> AGLIFS <mark>V</mark> PAAVYTTVV <mark>M</mark> HHDANDT	179
AD169	ATVALIAADRYRVLHKR <mark>T</mark> YARQSYRSTY <mark>M</mark> IL <mark>L</mark> LTW <mark>L</mark> AGLIFS <mark>V</mark> PAAVYTTVV <mark>MHHDANDT</mark>	179
FIX/PH/TR	ATVALIAADRYRVLHKR <mark>T</mark> YARQSYRSTY <mark>M</mark> IL <mark>L</mark> LTW <mark>L</mark> AGLIFS <mark>V</mark> PAAVYTTVV <mark>MHHDANDT</mark> ************************************	179
Merlin	<mark>QSSN</mark> GHATCVLYF <mark>I</mark> A <mark>D</mark> EV <mark>Y</mark> TVLLSWKVLLT <mark>L</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>A</mark> SQKQR	239
Towne	<mark>-NTN</mark> GHATCVLYF <mark>IAD</mark> EV <mark>Y</mark> TVLLSWKVLLT <mark>L</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>A</mark> SQKQR	238
Toledo	<mark>TNAT</mark> GHATCVLYF <mark>V</mark> A <mark>D</mark> EV <mark>H</mark> TVLLSWKVLLT <mark>L</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>T</mark> SQKQR	239
TB40/E	<mark>NNTN</mark> GHATCVLYF <mark>VAE</mark> EV <mark>H</mark> TVLLSWKVLLT <mark>L</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>T</mark> SQKQR	239
AD169	<mark>NNTN</mark> GHATCVLYF <mark>V</mark> A <mark>E</mark> EV <mark>H</mark> TVLLSWKVLLT <mark>M</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>T</mark> SQKQR	239
FIX/PH/TR	NNTNGHATCVLYF <mark>VAE</mark> EV <mark>H</mark> TVLLSWKVLLT <mark>L</mark> VWGAAPVIMMTWFYAFFYSTVQR <mark>T</mark> SQKQR .:.***********************************	239
Merlin	SRTLTFVSVLLISFVALQTPY <mark>V</mark> S <mark>I</mark> MIFNSYAT <mark>A</mark> AW <mark>PMD</mark> CEHLTLRRTIGTL <mark>S</mark> R <mark>L</mark> VPHLHC	299
Towne	SRTLTFVSVLLISFVALQTPY <mark>VSI</mark> MIFNSYAT <mark>A</mark> AW <mark>PMD</mark> CEHLTLRRTIGTL <mark>S</mark> R <mark>L</mark> VPHLHC	298
Toledo	SRTLTFVSVLLISFVALQTPY <mark>ISL</mark> MIFNSYAT <mark>T</mark> AW <mark>STK</mark> CEHLTLRRTIGTL <mark>A</mark> RLVPHLHC	299
TB40/E	SRTLTFVSVLLISFVALQTPY <mark>VSL</mark> MIFNSYAT <mark>T</mark> AW <mark>PMQ</mark> CEHLTLRRTIGTL <mark>ARV</mark> VPHLHC	299
AD169	SRTLTFVSVLLISFVALQTPY <mark>V</mark> S <mark>L</mark> MIFNSYAT <mark>T</mark> AW <mark>PMQ</mark> CEHLTLRRTIGTL <mark>A</mark> R <mark>V</mark> VPHLHC	299
FIX/PH/TR	SRTLTFVSVLLISFVALQTPY <mark>V</mark> SLMIFNSYAT <mark>T</mark> AW <mark>PMQ</mark> CEHLTLRRTIGTL <mark>A</mark> R <mark>V</mark> VPHLHC	299
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Merlin	LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL	359
Towne	LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL	358
Toledo	$\verb LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL $	359
TB40/E	LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL	359
AD169	LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL	359
FIX/PH/TR	LINPILYALLGHDFLQRMRQCFRGQLLDRRAFLRSQQNQRATAETNLAAGNNSQSVATSL ************************************	359
Merlin	D <mark>TS</mark> SKN <mark>C</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	412
Towne	D <mark>TN</mark> SKN <mark>C</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	411
Toledo	D <mark>PN</mark> SKN <mark>C</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	412
TB40/E	D <mark>TN</mark> SKN <mark>C</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	412
AD169	D <mark>TN</mark> SKN <mark>Y</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	412
FIX/PH/TR	D <mark>PN</mark> SKN <mark>C</mark> NQHAKRSVSFNFPSGTWKGGQKTASNDTSTKIPHRLSQSHHNLSGV	412
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Figure S2. Multiple sequence alignment of HCMV UL33 orthologs. Sequence variability is highlighted in yellow. (*) fully conserved residue; (:) conservation between groups of strongly similar properties; (.) conservation between groups of weakly similar properties.