

Figure S1. Target sequences in the nectin-2 knockout ex6 C7 cell clones. Schematic representation of the nectin-2, the edited nucleotide sequences, and the resulting amino acid sequences. Sequence analysis showed that the nectin-2 gene of ex6 C7-nec2-KO1 cells had a 2-nucleotide deletion in the signal sequence and that the ex6 C7-nec2-KO2 and-KO3 cells had a 1-nucleotide insertion, leading to the abolishment of a signal peptide and nectin-2 expression.

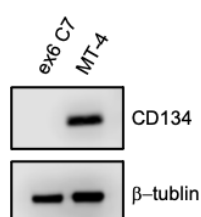


Figure S2. CD134 without the transmembrane anchor sequence was not detected inside the ex6 C7 cells. Ex6 C7 cells (7.5×10^5 cells) were washed with cold PBS, and then treated with 50 μ L of cold lysis buffer containing a protease inhibitor cocktail for 15 min at 4°C. Cell lysates were sonicated at 4°C for 10 s and centrifuged at $21,000 \times g$ for 10 min at 4°C. Aliquots of the supernatants (soluble fraction) were used for immunoblotting with anti-human CD134 monoclonal antibody (mAb) (clone: Ber-ACT35) and anti- β -tubulin mAb. MT-4 cells were used as the positive control.

A				
HSV-1_KOS-gD	1	KYALADASLKMADPNRFRGKDLVLDQLTDPGVRVYHIQAGLPDPFPQPSLPITVYAVLERACRSVLLNAPSEAPQIVRGASEDVVRKQPNLTIAWFRMGNGCAIPITVMEYTECSYKSLGACPIRTQPRWNYDFSFAVSEDNLG	150	
HSV-1_KOS_Rid1-gD	1P.....	150	
HSV-1_KOS_Rid2-gD	1R.....	150	
HSV-1_ANG-gD	1P.R.....	150	
HSV-2_333-gD	1P.....N.....R.....K.....PS.E.....I.....H.....DEA..HT.....Y...D.....P.....V.....S.....	150	
HSV-1_KOS-gD	151	FLMHAPAFETAGTYLRLVKINDWTEITQFILEHRAKGSCKYALPLRIPPSACLSPQAYQQGVTVDSIGMLPRFIPENQRTVAVYSLKIAGWHGPKAPYTSTLLPPELSETPNATQPELAPEDPEDSALLEDPVGTVPQIPPNWHIPSIQ	300	
HSV-1_KOS_Rid1-gD	151	300	
HSV-1_KOS_Rid2-gD	151	300	
HSV-1_ANG-gD	151I.....	300	
HSV-2_333-gD	151RA.....A..TSK.....L.....P.....D.T.....V.....A..SS.....	300	
HSV-1_KOS-gD	301	DAATPYHPPATPNMGLIAGAVGSSLAALVICGIVYWMHRRTRKAPKRIRLPHIREDDQPSHHQPLFY	369	
HSV-1_KOS_Rid1-gD	301	369	
HSV-1_KOS_Rid2-gD	301R..Q.G.....	369	
HSV-1_ANG-gD	301	369	
HSV-2_333-gD	301	.V..-H.A..A.S.P..I..LA..T..V...G..AF.VR..AQM...L.....D..A.P.....	368	
B				
HHV-68_HST-gB	1	SDNYIRAGYNHYPFRICSIAGKTDLMRFRDISCSYKSNAMSEGGFFIIYKNTIETTFPVRTYKNELTFQTSYRDVGCVYFLDRTVMGLAMPVYEANLVNSRAQCYSAVAIRKPDGTVFSAIHEDNNKNETLELFFLNFKSVTNKRF	150	
HHV-68_Z29-gB	1	..D.....	150	
HHV-68_HST-gB	151	ITTKEPYFARGPLWLYSTSLNCIVTEAMAKAYPFYSFALTTEIVEGSPFFDGSNGKHFAPLEKLTILENYTMIEDLMNGMGATTLVKRIAFLEKGDTLFSWEIKEENESVCMKHMTTTHGLRAETDETYHFIISKELTAAFTV	300	
HHV-68_Z29-gB	151T.....K.....A.....	300	
HHV-68_HST-gB	301	PKDSLNLTPDKQTCIKNEFENIKEYVMSYNDYTMNGSYQIFKTTGDLILIWQPLVQKSLMVLEQGSVNLRRRDLVDVKSRRHILYVQLQYLDTLKDYNALGNLAESWCLDQKRTITMLHLSKISPSIVSEVYGRPISAGLH	450	
HHV-68_Z29-gB	301	S.E.....K..TD.....	450	
HHV-68_HST-gB	451	GDVLAISKIEVNQSSVQLHKSRRVDAKGLRSETMTCYNRPLVTFSEVNSTPEVVPQGLDNEILLGDHRTREECEIPSTKIFLSGNHAHVYDYTHNTSTPIEDIEVLDAFIRLKIIDPLENADFKLLDLYSPDELSRANVFDLENILRE	600	
HHV-68_Z29-gB	451V.....	600	
HHV-68_HST-gB	601	YNSYKSALYTIEAKIATNTPSYVNGINSFLQGLAIGTGLGSVISVTAGALGDIVGGVVSFLKNPFGGGLMLILAIIVVVVIIIVVFVRQKHVLSKPIDMMFPYATNPVTVSSVTGTTVVKTSPVKDADGGTSVAVSEKEEGMADVSGQI	750	
HHV-68_Z29-gB	601T.....	750	
HHV-68_HST-gB	751	SGDEYSQEDALKMLKAISLDESRYRRKPSSSESHASKPSLIDIRIRYRGYKSVNVEEA	807	
HHV-68_Z29-gB	751	807	

Figure S3. Comparison of the gD and gB amino acid sequences. (A) gD amino acid sequences in the HSV-1 strain KOS, HSV-1 mutant strains, and HSV-2 strain 333. The 25-amino acid signal sequence is excluded. The amino acid substitutions (L25P, Q27P, and Q27R) in HSV-1 that could be important for entry in nectin-2 expressing cells are identified in red (Dean *et al.*, 1994. *Virology*, Lopez *et al.*, 2000. *J Virol*). (B) gB amino acid sequences in HHV-6B strains HST and Z29. The 31-amino acid signal sequence is excluded.