

Supplementary Figure 1: SplitsTree analysis of KOS79 and KOS63-like variants.

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SplitsTree analysis (44, 45, 70) reveals the close relationship among the KOS63-like variants, as compared to either KOS79 or the HSV-1 reference strain 17. Inset (green box) provides an expanded view and labels for the compact section of the network containing KOS63-like strains. Trimmed genome sequences lacking the terminal copies of the large repeats that flank each unique region were excluded, so that these genome regions did not doubly influence the genetic relatedness. Likewise, reiterations set to match those of strain 17 were excluded, so that these did not unduly influence the network (see Methods for details). Genome accession numbers and sources are listed in Table 1.

Supplementary Figure 2: Simplot and BootScan comparison of KOS79 with



consensus of the European/North American and Africa-1 clusters.

Viral forensic genomics reveals the relatedness of classic herpes simplex virus strains KOS, KOS63, and KOS79 Supplementary Material Bowen *et al*, Virology, 2016.

Supplementary Figure 2: Simplot and BootScan comparison of KOS79 with consensus of the European/North American and Africa-1 clusters.

SimPlot analysis (**A**) of KOS79, in comparison to KOS63, a consensus of the European/North American cluster (strains HF10, 17, McKrae, F, and H129), and a consensus of the Africa-1 cluster (strains E08, E12, E13, E14, and E19) from our prior study (Figure 2) (42). KOS79 is most closely related to the European/North American consensus strain over most of its length, except for a few short regions where it is most similar to the Africa-1 consensus (e.g. between kilobases 45-50 and 97-102) or where no predominant background can be identified (e.g. between kilobases 60-65 and 80-85). These regions may represent recombination events, although whether recent or ancient cannot be known. The corresponding BootScan plot (**B**) shows chi-shaped crossover signals supportive of recombination with an African strain at ~97-102 kb, although two other areas of African similarity have less well formed signs of crossover. Potential crossover events with KOS63 occur at 60-60 kb and 80-85 kb (**B**), although the SimPlot signals in these areas are not strong (**A**).

Supplementary Table 1: Proteins varying between each pairwise combination of

KOS63-like strains.

Strains	KOS63	KOS _{Davido}	KOS _{Large}	KOS Kinchington	KOS _{Knipe}
KOS _{Davido}	gB (UL27), VP1/2 (UL36), RR1 (UL39), ICP4 (RS1)	-	-	-	-
KOS _{Large}	UL8, UL9, UL13, UL21, gH (UL22), VP1/2 (UL36), US6	UL8, UL9, UL13, UL21, gH (UL22), gB (UL27), VP1/2 (UL36), RR1 (UL39), ICP4 (RS1), gD (US6)	-	-	-
KOS Kinchington	gB (UL27), VP1/2 (UL36), RR1 (UL39)	VP1/2 (UL36), ICP4 (RS1)	UL8, UL9, UL13, UL21, gH (UL22), gB (UL27), VP1/2 (UL36), RR1 (UL39), gD (US6)	-	-
KOS _{Knipe}	gB (UL27), VP1/2 (UL36), RR1 (UL39), ICP34.5 (RL1), ICP0 (RL2)	ICP4 (RS1), VP1/2 (UL36), ICP34.5 (RL1), ICP0 (RL2)	UL8, UL9, UL13, UL21, gH (UL22), gB (UL27), VP1/2 (UL36), RR1 (UL39), ICP34.5 (RL1), ICP0 (RL2), gD (US6)	VP1/2 (UL36), ICP34.5 (RL1), ICP0 (RL2)	-
KOS1.1	UL30, VP1/2 (UL36)	gB (UL27), UL30, VP1/2 (UL36), RR1 (UL39), ICP4 (RS1)	UL8, UL9, UL13, UL21, gH (UL22), UL30, VP1/2 (UL36), gD (US6)	gB (UL27), UL30, VP1/2 (UL36), RR1 (UL39)	gB (UL27), UL30, VP1/2 (UL36), RR1 (UL39), ICP34.5 (RL1), ICP0 (RL2)

Primer Name	Target	Sequence
KOS_UL14_F1	UL14	5'-GGGGAACACTATCTGTCGTTGTTGCAGC-3'
KOS_UL14_R1	UL14	5'-GATCGTCTTGCGGACCAGGAGGAGCAA-3'
KOS_UL14_F2_Seq	UL14	5'-GCGTGAGGGTAAGGATGTG-3'
KOS_UL14_F2.1_Seq	UL14	5'-GCGTGAGGGTGAGGATGTG-3'
KOS_UL14_R2_Seq	UL14	5'-CTGGTCATGTGGCAGCTAA-3'
KOS_UL25_F1	UL25	5'-GTCGATATCGAGCGCCGGTTAC-3'
KOS_UL25_R1	UL25	5'-GGTACAGCAGGTAGAGACACAACAC-3'
KOS_UL25_F2_Seq	UL25	5'-CGGAACGTGCACGAGAT-3'
KOS_UL25_R2_Seq	UL25	5'-CGTCATGAAGGTCTTGGACA-3'

Supplementary Table 2: Primers for PCR Validation