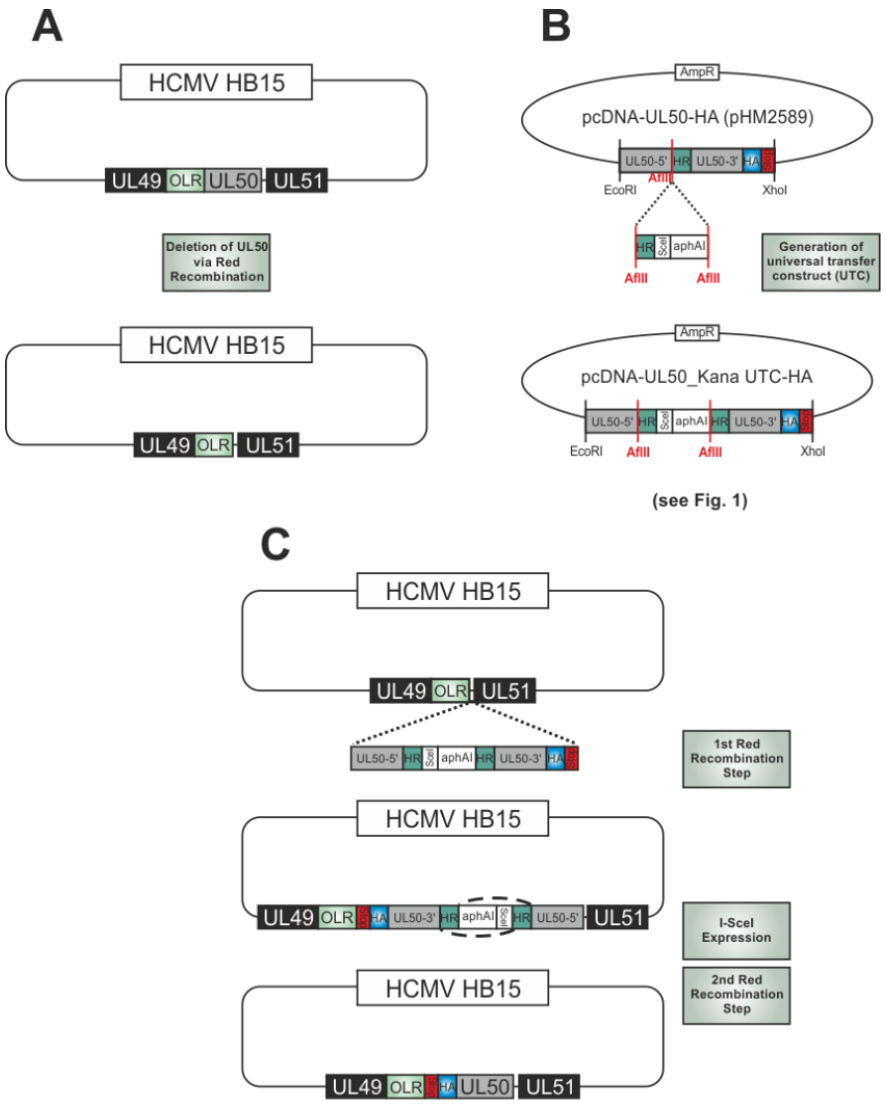

Figure S1. Schematic representation of the generation of recombinant HCMVs. **(A)** pUL50 was partially deleted from the HB15 BACmid using Red recombination without affecting the pUL49/pUL50 overlapping region (OLR, highlighted in light green). **(B)** Generation of UTCs, here depicted for wild-type pUL50-HA. Constructs containing homologous regions (HR) important for the second step of Red recombination, an I-SceI restriction site and a kanamycin cassette (aphAI) were inserted into pcDNA3.1 plasmids harboring pUL50-HA, pUL50(S216A)-HA, pUL50(Px5)-HA, pUL50(Mx5)-HA, pUL50, pUL50(Px14), pUL50(Mx14) via an AflII restriction site. The HA-tag is highlighted in blue and the stop codon in red. **(C)** Exemplary insertion of wild-type ORF UL50-HA (Rev1) Kana into UL50-deleted HB15 HB15/AD169ΔUL50. Prior to transformation, homologous regions for insertion into the BACmid were amplified in the respective UTCs and subsequently transformed into bacteria containing HB15/AD169ΔUL50 [19]. After I-SceI induction, the second step of Red recombination occurred at homologous regions (HRs, dashed lines) and the kanamycin cassette was deleted from the backbone resulting in the WT pUL50-HA (Rev1) construct.

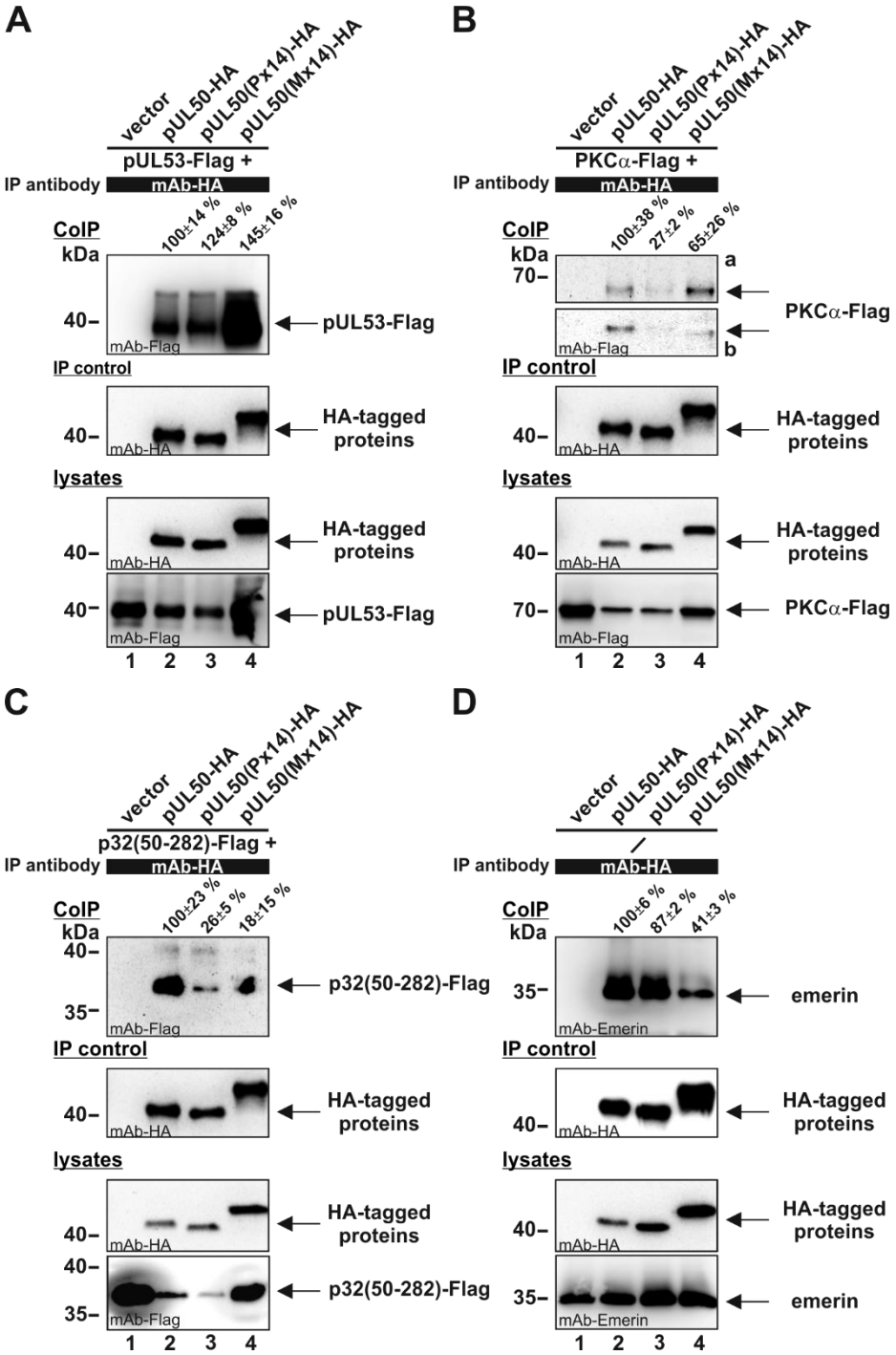

Figure S2. CoIP-based interaction analysis of pUL50 phophosite mutants. 293T cells were transiently transfected with expression plasmids coding for HA-tagged pUL50, pUL50(Px14) or pUL50(Mx14) and Flag-tagged pUL53, PKCα or p32(50-282). At three d p.t., cells were lysed and HA-tagged proteins were immunoprecipitated using mAb-HA. Lysate controls taken prior to the IP and CoIP samples were subjected to standard Wb analysis using tag-specific antibodies as indicated. Signal intensities of pUL50 interaction partners pUL53 **(A)**, PKCα **(B)**, p32(50-282) **(C)** and emerin **(D)** were quantified using Aida Image Analyzer v.4.23.

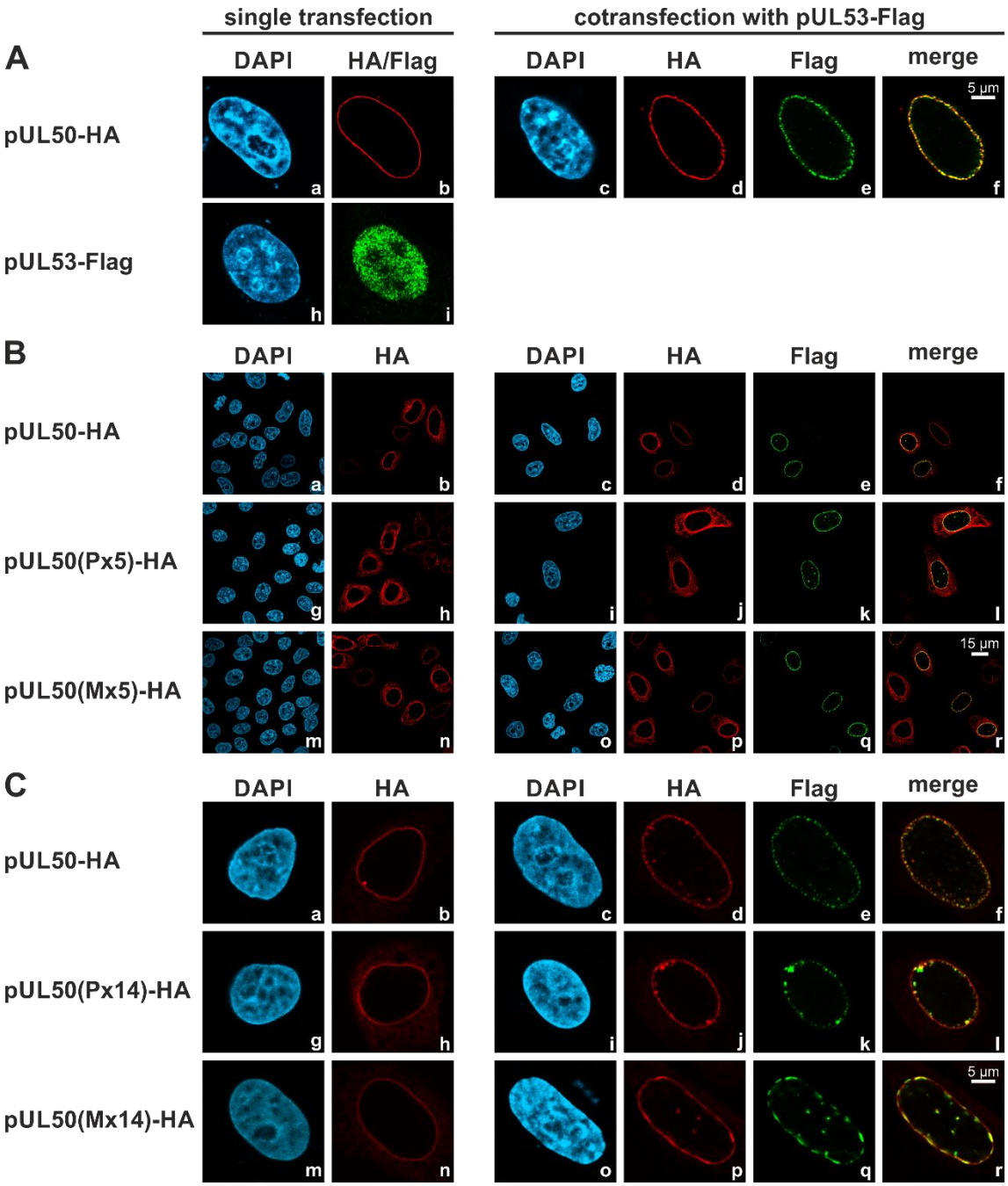

Figure S3. Confocal imaging analysis of the localization of pUL50 and pUL53 of transfected phosphosite mutants. HeLa cells were transiently transfected with constructs coding for pUL50-HA, pUL53-Flag, pUL50(Px5)-HA, pUL50(Mx5)-HA, pUL50(Px14)-HA, pUL50(Mx14)-HA (left panel) or cotransfected with pUL53-Flag (right panel). Two d p.t., cells were fixed and used for immunostaining with tag-specific antibodies analyzed by confocal imaging. **(A)** WT constructs for pUL50 and pUL53. **(B)** Phosphosite mutants with five exchanged phosphosites. **(C)** Phosphosite mutants with 14 exchanged phosphosites.

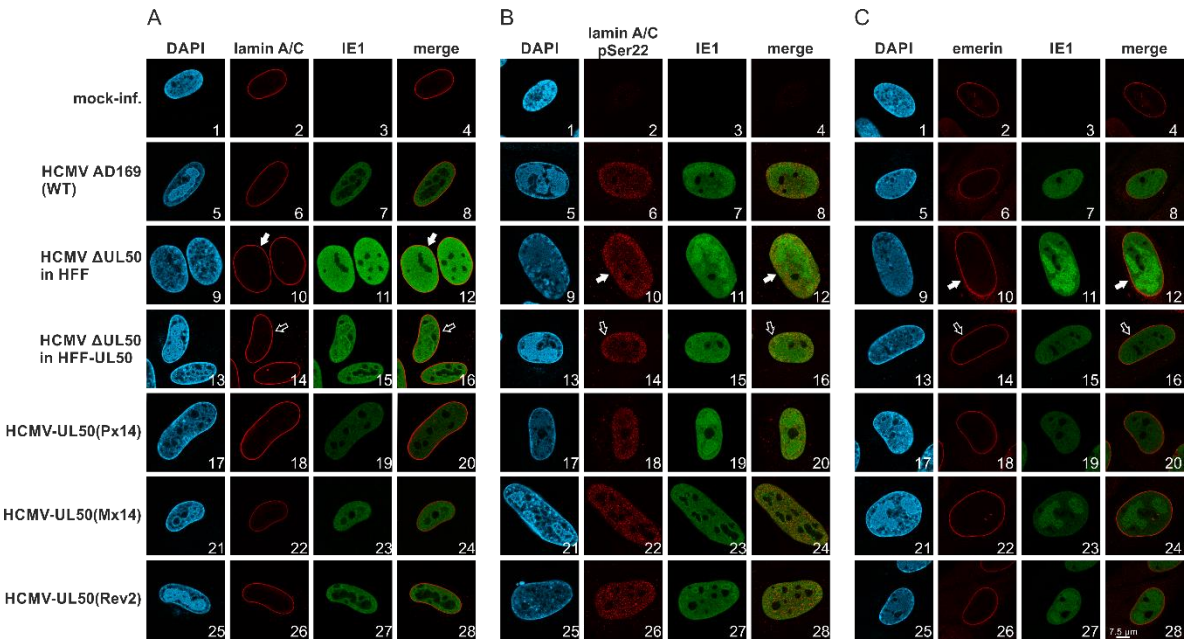

Figure S4. Confocal imaging analysis of the localization of cellular proteins produced by HCMV ΔUL50 or pUL50 phosphosite mutations. Normal HFFs or pUL50-complementing cells (HFF-UL50 +Dox) were used for infection with the recombinant HCMVs at a MOI of 0.05 and harvested at 7 d p.i.. Immunofluorescence staining was performed with antibodies against the indicated proteins and representative panels of confocal imaging are given (see scale bar in panel C, picture 28). **(A-C)** Cellular proteins lamin A/C, lamin A/C pSer22 and emerin, showing no alteration in localization patterns.

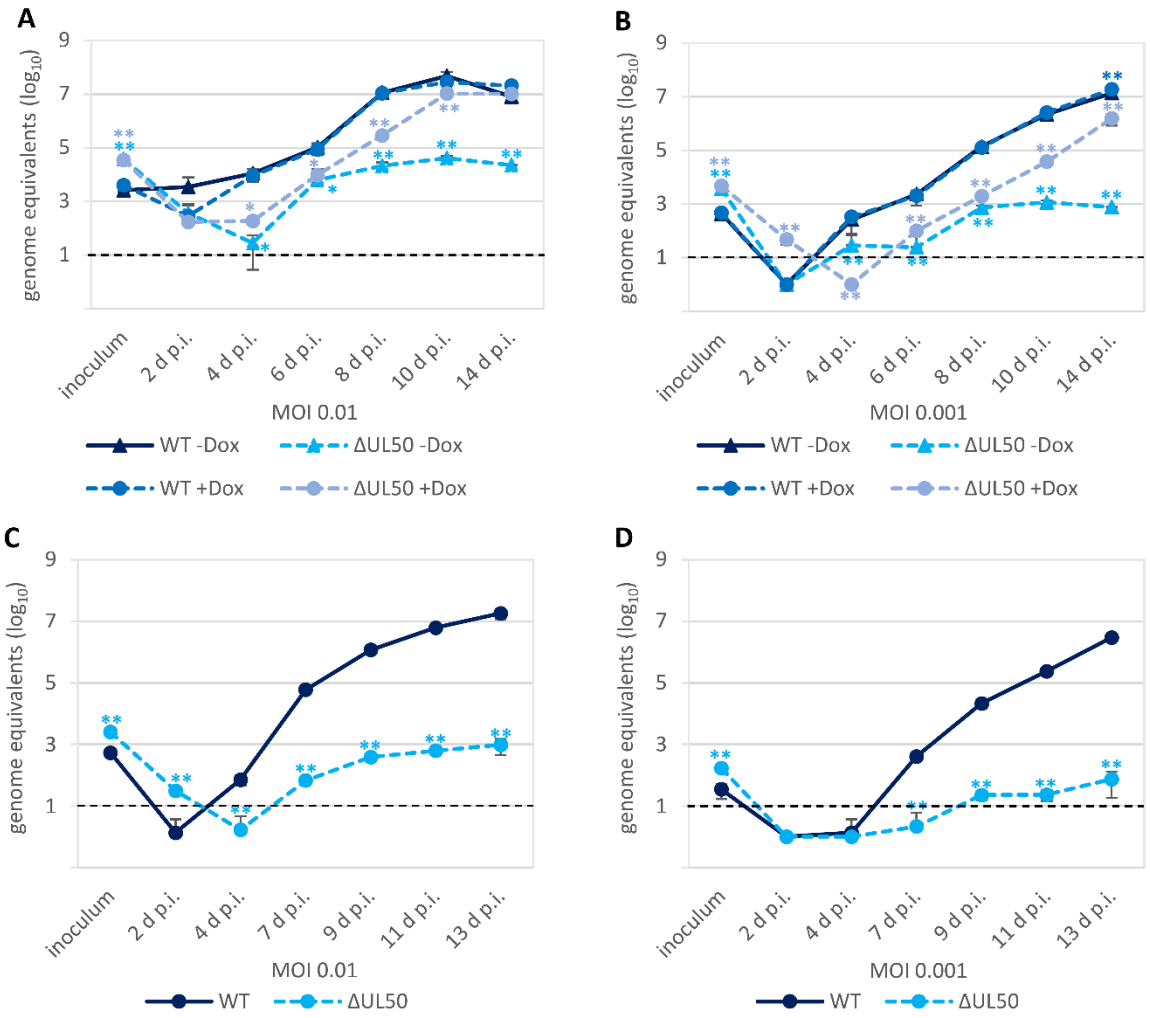

Figure S5. HCMV replication kinetics of the ORF-UL50 deletion mutant. HFF-UL50 cells **(A, B)** or HFFs **(C, D)** were infected with parental HCMV AD169 (WT) or recombinant HCMV ΔUL50 at a MOI of 0.01 **(A, C)** or 0.001 **(B, D)**. Viral supernatants were harvested at the indicated time point and viral genome equivalents released into the supernatant were determined by IE1-specific quantitative real-time PCR. Each infection was performed in triplicate; mean values and standard deviations are shown. The significance is calculated relating to WT (solid lines). The black dashed line shows the limit of detection. *, $p \leq 0.05$; **, $p \leq 0.01$

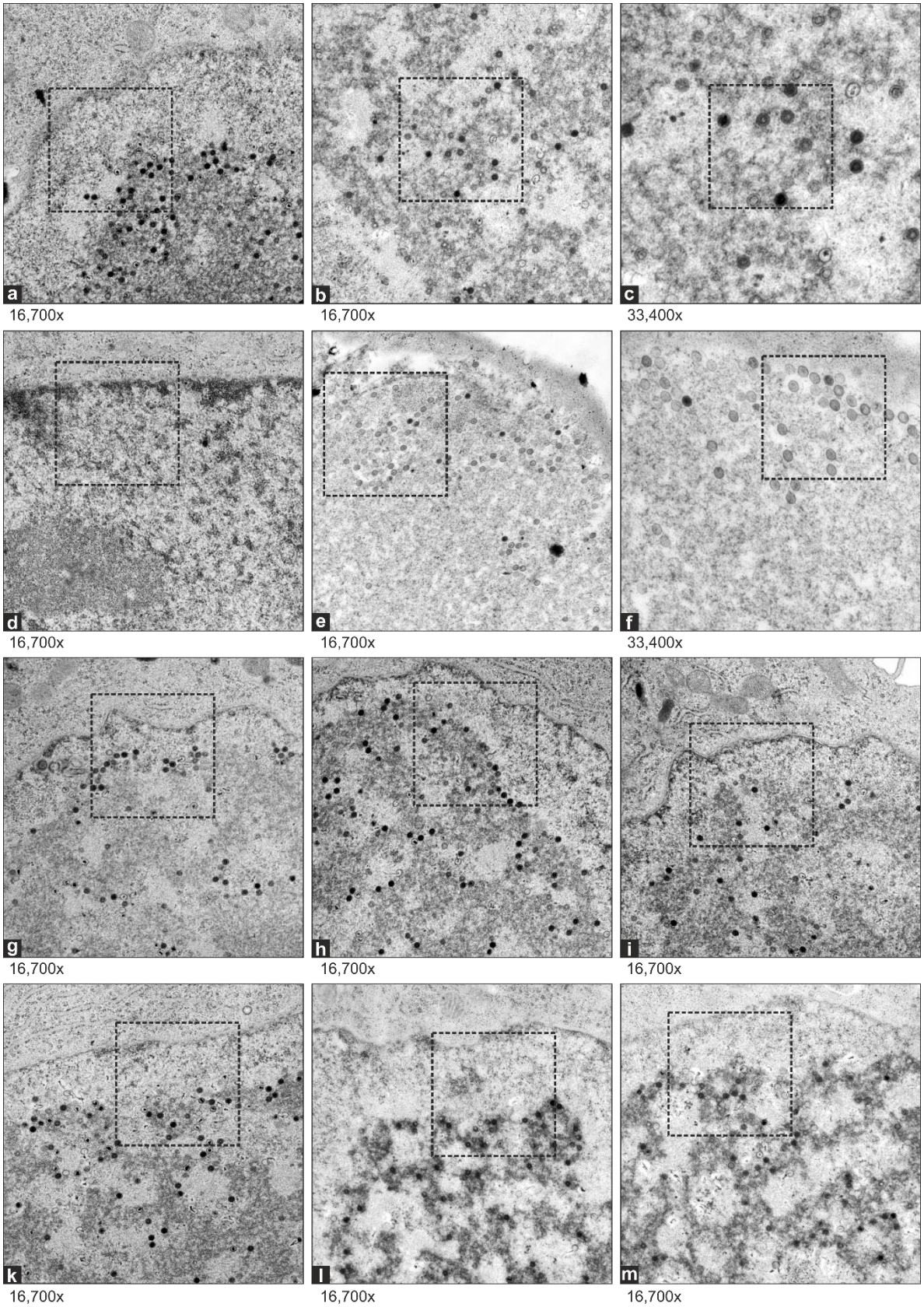

Figure S6. Electron microscopic investigation of recombinant viruses. HFFs or HFF-UL50 (b,c) remained mock-infected (d) or were infected with WT (a), ΔUL50 +Dox (b,c), ΔUL50 –Dox (e,f), Rev1 (g), Px5 (h), Mx5 (i), S216A (k), Px14 (l) or Mx14 (m) and were fixed at 4-7 d p.i. Samples were analyzed by TEM; magnification as indicated. Dashed boxes indicate enlarged pictures shown in Figure 7.

A

UL50 Sequences

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WT        MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS 60
ΔUL50     ------------------------------------------------------------
Px5       MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS
Mx5       MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS
Rev1      MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS
Px14      MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS
Mx14      MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS
Rev2      MEMNKVLHQDLVQATRRILKLGPSELRVTDAGLICKNPNYSVCDAMLKTDTVYCVEYLLS

WT        YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVSPARHVGEFNVLKVNESLIVTLKDIE 120
ΔUL50     ------------------------------------------------------------
Px5       YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVSPARHVGEFNVLKVNESLIVTLKDIE
Mx5       YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVSPARHVGEFNVLKVNESLIVTLKDIE
Rev1      YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVSPARHVGEFNVLKVNESLIVTLKDIE
Px14      YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVAPARHVGEFNVLKVNESLIVTLKDIE
Mx14      YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVEPARHVGEFNVLKVNESLIVTLKDIE
Rev2      YWESRTDHVPCFIFKNTGCAVSLCCFVRAPVKLVSPARHVGEFNVLKVNESLIVTLKDIE

WT        EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASTSLAVRN 180
ΔUL50     ------------------------------------------------------------
Px5       EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASTSLAVRN
Mx5       EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASTSLAVRN
Rev1      EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASTSLAVRN
Px14      EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASASLAVRN
Mx14      EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASESLAVRN
Rev2      EIKPSAYGVLTKCVVRKSNSASVFNIELIAFGPENEGEYENLLRELYAKKAASTSLAVRN

WT        HVTVSSHSGSGPSLWRARMSAALTRTAGKRSSRTASPPPPPRHPSCSPTMVAAGGAAAGP 239
ΔUL50     ------------------------------------------------------------
Px5       HVTVSSHAGSGPSLWRARMSAALTRTAGKRSSRAAAPPPPPRHPSCSPTMVAAGGAAAGP
Mx5       HVTVSSHEGSGPSLWRARMSAALTRTAGKRSSREAEPPPPPRHPSCSPTMVAAGGAAAGP
Rev1      HVTVSSHSGSGPSLWRARMSAALTRTAGKRSSRTASPPPPPRHPSCSPTMVAAGGAAAGP
Px14      HVAVSSHAGSGPSLWRARMSAALTRTAGKRSSRAAAPPPPPRHPACAPAMVAAGGAAAGP
Mx14      HVEVSSHEGSGPSLWRARMSAALTRTAGKRSSREAEPPPPPRHPECEPEMVAAGGAAAGP
Rev2      HVTVSSHSGSGPSLWRARMSAALTRTAGKRSSRTASPPPPPRHPSCSPTMVAAGGAAAGP
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WT          RPPPPPMAAGSWRLCRCEACMGRCGCASEGDADEEEEELLALAGEGKAAAAAAGQDVGGS 299
ΔUL50       ------------------------------------------------------------
Px5         RPPPPPMAAGSWRLCRCEACMGRCGCASEGDADEEEEELLALAGEGKAAAAAAGQDVGGA
Mx5         RPPPPPMAAGSWRLCRCEACMGRCGCASEGDADEEEEELLALAGEGKAAAAAAGQDVGGE
Rev1        RPPPPPMAAGSWRLCRCEACMGRCGCASEGDADEEEEELLALAGEGKAAAAAAGQDVGGS
Px14        RPPPPPMAAGAWRLCRCEACMGRCGCAAEGDADEEEEELLALAGEGKAAAAAAGQDVGGA
Mx14        RPPPPPMAAGEWRLCRCEACMGRCGCAEEGDADEEEEELLALAGEGKAAAAAAGQDVGGE
Rev2        RPPPPPMAAGSWRLCRCEACMGRCGCASEGDADEEEEELLALAGEGKAAAAAAGQDVGGS

WT          ARRPLEEHVSRRRGVSTHHRHPPSPPCAPSLERTGYRWAPSSWWRARSGPSRPQSGPWLP 359
ΔUL50       ------------------------------------------------------------
Px5         ARRPLEEHVSRRRGVSTHHRHPPAPPCAPSLERTGYRWAPSSWWRARSGPSRPQSGPWLP
Mx5         ARRPLEEHVSRRRGVSTHHRHPPEPPCAPSLERTGYRWAPSSWWRARSGPSRPQSGPWLP
Rev1        ARRPLEEHVSRRRGVSTHHRHPPSPPCAPSLERTGYRWAPSSWWRARSGPSRPQSGPWLP
Px14        ARRPLEEHVSRRRGVSTHHRHPPAPPCAPALERTGYRWAPSSWWRARSGPSRPQSGPWLP
Mx14        ARRPLEEHVSRRRGVSTHHRHPPEPPCAPELERTGYRWAPSSWWRARSGPSRPQSGPWLP
Rev2        ARRPLEEHVSRRRGVSTHHRHPPSPPCAPSLERTGYRWAPSSWWRARSGPSRPQSGPWLP

WT          ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRD---------        396
ΔUL50       ------------------------GQSSSPTRSAHRD--------
Px5         ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRDYPYDVPDYA
Mx5         ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRDYPYDVPDYA
Rev1        ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRDYPYDVPDYA
Px14        ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRD---------
Mx14        ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRD---------
Rev2        ARFATLGPLVLALLLVLALLWRGHGQSSSPTRSAHRD---------
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B

UL53 Sequences

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ΔUL50       MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Px5         MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Mx5         MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Rev1        MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Px14        MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Mx14        MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
Rev2        MSSVSGVRTPRERRSALRSLLRKRRQRELASKVASTVNGATSANNHGEPPSPADARPRLT
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WT          LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI 120
ΔUL50       LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
Px5         LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
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Mx5         LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
Rev1        LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
Px14        LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
Mx14        LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
Rev2        LHDLHDIFREHPELELKYLNMMKMAITGKESICLPFNFHSHRQHTCLDISPYGNEQVSRI
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WT          ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL 180
ΔUL50       ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Px5         ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Mx5         ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Rev1        ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Px14        ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Mx14        ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
Rev2        ACTSCEDNRILPTASDAMVAFINQTSNIMKNRNFYYGFCKSSELLKLSTNQPPIFQIYYL
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WT          LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV 240
ΔUL50       LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Px5         LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Mx5         LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Rev1        LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Px14        LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Mx14        LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
Rev2        LHAANHDIVPFMHAEDGRLHMHVIFENPDVHIPCDCITQMLTAAREDYSVTLNIVRDHVV
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WT          ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT 300
ΔUL50       ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Px5         ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Mx5         ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Rev1        ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Px14        ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Mx14        ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
Rev2        ISVLCHAVSASSVKIDVTILQRKIDEMDIPNDVSESFERYKELIQELCQSSGNNLYEEAT
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ΔUL50     SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Px5       SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Mx5       SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Rev1      SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Px14      SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Mx14      SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
Rev2      SSYAIRSPLTASPLHVVSTNGCGPSSSSQSTPPHLHPPSQATQPHHYSHHQSQSQQHHHR
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WT        PQSPPPPLFLNSIRAP     375
ΔUL50     PQSPPPPLFLNSIRAP
Px5       PQSPPPPLFLNSIRAP
Mx5       PQSPPPPLFLNSIRAP
Rev1      PQSPPPPLFLNSIRAP
Px14      PQSPPPPLFLNSIRAP
Mx14      PQSPPPPLFLNSIRAP
Rev2      PQSPPPPLFLNSIRAP
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C

UL97 Sequences

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ΔUL50     MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Px5       MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Mx5       MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Rev1      MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Px14      MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Mx14      MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
Rev2      MSSALRSRARSASLGTTTQGWDPPPLRRPSRARRRQWMREAAQAAAQAAVQAAQAAAAQV
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WT        AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA 120
ΔUL50     AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Px5       AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Mx5       AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Rev1      AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Px14      AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Mx14      AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
Rev2      AQAHVDENEVVDLMADEAGGGVTTLTTLSSVSTTTVLGHATFSACVRSDVMRDGEKEDAA
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WT          SDKENLRRPVVPSTSSRGSAASGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS 180
ΔUL50       SDKENLRRPVVPSTSSRGSAASGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Px5         SDKENLRRPVVPSTSSRGSAAGGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Mx5         SDKENLRRPVVPSTSSRGSAAGGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Rev1        SDKENLRRPVVPSTSSRGSAAGGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Px14        SDKENLRRPVVPSTSSRGSAASGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Mx14        SDKENLRRPVVPSTSSRGSAASGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
Rev2        SDKENLRRPVVPSTSSRGSAASGDGYHGLRCRETSAMWSFEYDRDGDVTSVRRALFTGGS
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WT          DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG 240
ΔUL50       DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Px5         DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Mx5         DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Rev1        DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Px14        DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Mx14        DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
Rev2        DPSDSVSGVRGGRKRPLRPPLVSLARTPLCRRRVGGVDAVLEENDVELRAESQDSAVASG
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WT          PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP 300
ΔUL50       PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Px5         PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Mx5         PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Rev1        PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Px14        PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Mx14        PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
Rev2        PGRIPQPLSGSSGEESATAVEADSTSHDDVHCTCSNDQIITTSIRGLTCDPRMFLRLTHP
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WT          ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH 360
ΔUL50       ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Px5         ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Mx5         ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Rev1        ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Px14        ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Mx14        ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
Rev2        ELCELSISYLLVYVPKEDDFCHKICYAVDMSDESYRLGQGSFGEVWPLDRYRVVKVARKH
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WT        SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH 420
ΔUL50     SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Px5       SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Mx5       SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Rev1      SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Px14      SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Mx14      SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
Rev2      SETVLTVWMSGLIRTRAAGEQQQPPSLVGTGVHRGLLTATGCCLLHNVTVHRRFHTDMFH
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WT        HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC 480
ΔUL50     HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Px5       HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Mx5       HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Rev1      HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Px14      HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Mx14      HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
Rev2      HDQWKLACIDSYRRAFCTLADAIKFLNHQCRVCHFDITPMNVLIDVNPHNPSEIVRAALC
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WT        DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF 540
ΔUL50     DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Px5       DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Mx5       DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Rev1      DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Px14      DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Mx14      DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
Rev2      DYSLSEPYPDYNERCVAVFQETGTARRIPNCSHRLRECYHPAFRPMPLQKLLICDPHARF
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WT        PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL 600
ΔUL50     PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Px5       PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Mx5       PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Rev1      PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Px14      PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Mx14      PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
Rev2      PVAGLRRYCMSELSALGNVLGFCLMRLLDRRGLDEVRMGTEALLFKHAGAACRALENGKL
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WT          THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM 660
ΔUL50       THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Px5         THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Mx5         THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Rev1        THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Px14        THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Mx14        THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
Rev2        THCSDACLLILAAQMSYGACLLGEHGAALVSHTLRFVEAKMSSCRVRAFRRFYHECSQTM
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WT          LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE 706
ΔUL50       LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Px5         LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Mx5         LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Rev1        LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Px14        LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Mx14        LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
Rev2        LHEYVRKNVERLLATSDGLYLYNAFRRTTSIICEEDLDGDCRQLFPE
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Figure S7: Alignments of pUL50, pUL53 and pUL97 of recombinant viruses. Amino acid sequences of reconstituted viruses for pUL50 **(A)** pUL53 **(B)** and pUL97 **(C)**. Phosphosite mutations are highlighted in dark grey, additional mutations in bright grey. * identical amino acids, - no sequence.