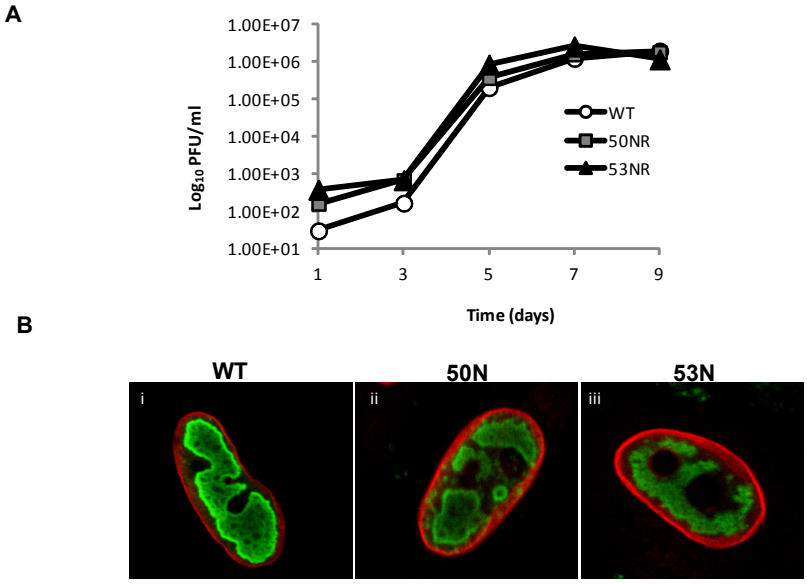
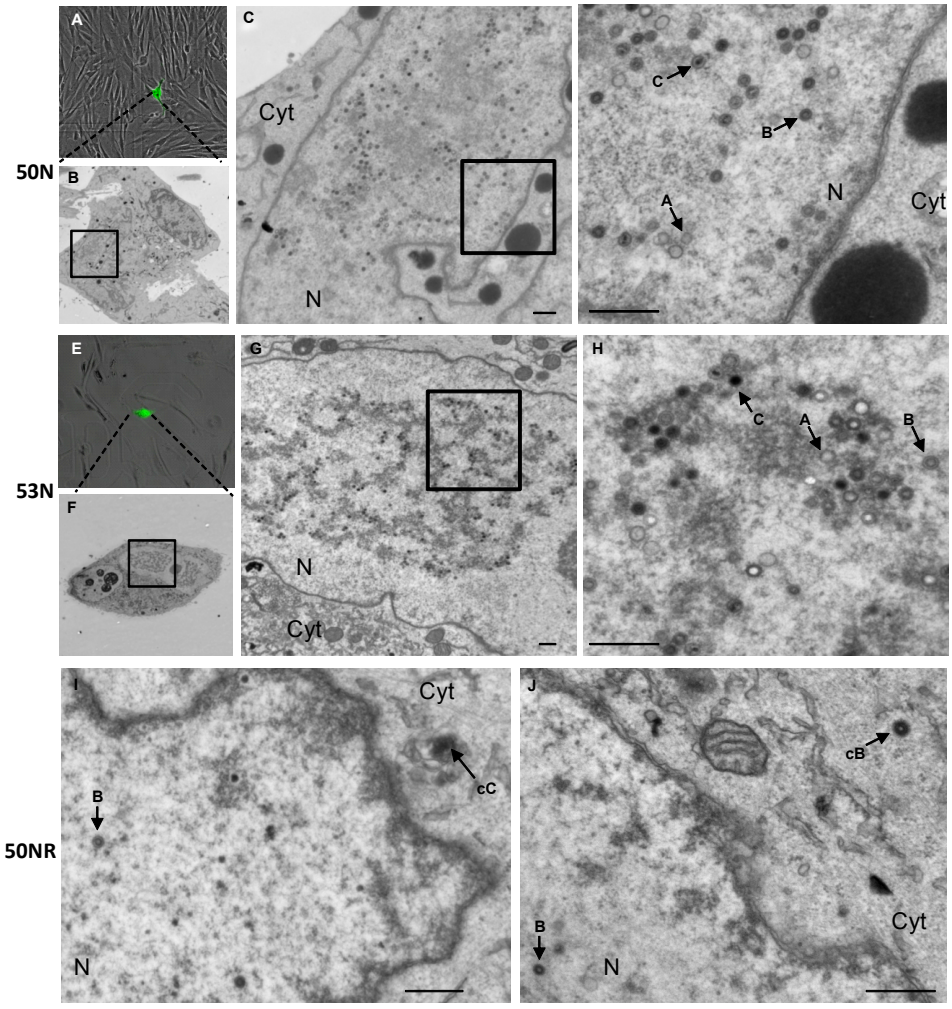
Supplemental Table S1. Primer sequences for HCMV AD169 BAC or BADGFP constructs

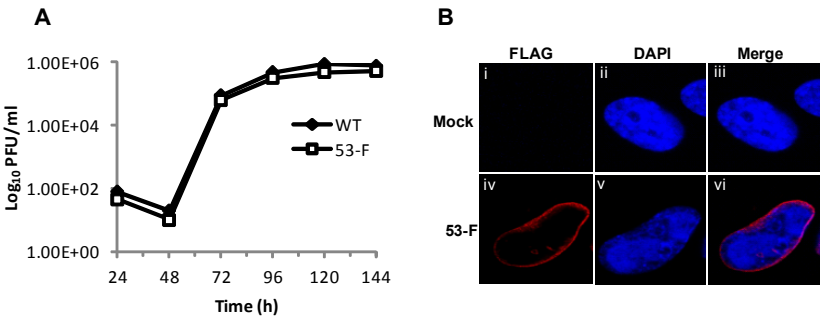
Construct	Primer	Sequence
UL50 Null	UL50 Null Fw	CATCTGTAAAAACCCCAATTACTCGGTGTGCGACTGATAATAGAAAGAC AGACACGGTCTATTAGGGATAACAGGGTAATCG
	UL50 Null Rv	CTGAGTAGATACTCGACACAATAGACCGTGTCTGTCTTTCTATTATCAGT CGCACACCGAGTAATGCCAGTGTTACAACCAATTAAC
UL53 Null	UL53 Null Fw	GCGAGCACCCCGAACTGGAGCTCAAGTACCTTAACTGATAGAAGTAGC CATCACGGGCAAAGAGTTAGGGATAACAGGGTAATCG
	UL53 Null Rv	GAAGGGTAAGCAGATGGACTCTTTGCCCGTGATGGCTACTTCTATCAGT TAAGGTACTTGAGCTCgCCAGTGTTACAACCAATTAAC
UL50 Null Rescue	UL50 Null res Fw	CATCTGTAAAAACCCCAATTACTCGGTGTGCGACGCCATGCTCAAGACA GACACGGTCTATTAGGGATAACAGGGTAATCG
	UL50 Null res Rv	CTGAGTAGATACTCGACACAATAGACCGTGTCTGTCTTGAGCATGGCGT CGCACACCGAGTAATGCCAGTGTTACAACCAATTAAC
UL53 Null Rescue	UL53 Null res Fw	GCGAGCACCCCGAACTGGAGCTCAAGTACCTTAACATGATGAAGATGG CCATCACGGGCAAAGAGTTAGGGATAACAGGGTAATCG
	UL53 Null res Rv	GAAGGGTAAGCAGATGGACTCTTTGCCCGTGATGGCCATCTTCATCAT GTTAAGGTACTTGAGCTCGCCAGTGTTACAACCAATTAAC
FLAG-UL97	FLAG-UL97 Fw	CAGCCTTAGGAACAGGGAAGACTGTCGCCACTATGGACTACAAGGATG ACGACGATAAGTCCTCCGCACTTCGGTTAGGGATAACAGGGTAATCG
	FLAG-UL97 Rv	GAGGCCGAGCGAGCCCGAGACCGAAGTGCGGAGGACTTATCGTCGTC ATCCTTGTAGTCCATAGTGGCGACAGTCGCCAGTGTTACAACCAATTAA C
UL53-FLAG	53CTFa1 Fw	TGTTTCTGAATTCCATCAGGGCCCCAGACTACAAGGATGACGACGATAA GTAGCTCAACAGCTAGGGATAACAGGGTAATCGATTT
	53CTFa1 Rv	GCACGAATGCTGTTGAGCTACTTATCGTCGTCATCCTTGTAGTCTGGGG CCCTGATGGAATTCAGAAACAGCGGGCCAGTGTTACAACCAATTAAC
	53CTF p2 Fw	GCATCATCACCGTCCCCAGTCACCACCGCCGCCGCTGTTTCTGAATTC CATCAG
	53CTF p2 Rv	TTTCTGCCGTACAGTGTCAAGGCGCACGAATGCTGTTGAGCTA



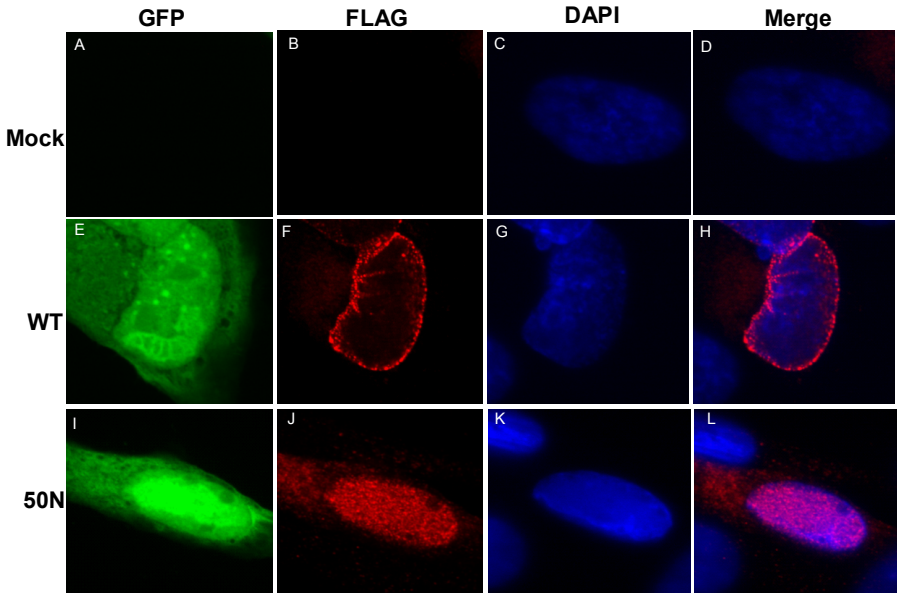
Suppplemental Figure S1. A. Multiple-cycle growth kinetics of the rescued derivatives. Rescued derivatives for *UL50* or *UL53* null constructs, 50NR and 53NR BADGFP, were compared with WT BADGFP virus in HFF cells in 24-well plates (MOI of 0.1 PFU/cell). Titers were calculated by averaging plaque counts from triplicate titrations. Because counts from three sets of titrations differed less than twofold for all data points, error bars are not shown. **B. Replication compartment formation by the null mutants**. HFF cells electroporated with WT (i), *UL50* null (50N, panel ii) or *UL53* null (53N, panel iii) pBADGFP constructs, were fixed at day 7 and stained for lamin A/C (red) and UL44 (green) and visualized using confocal microscopy.



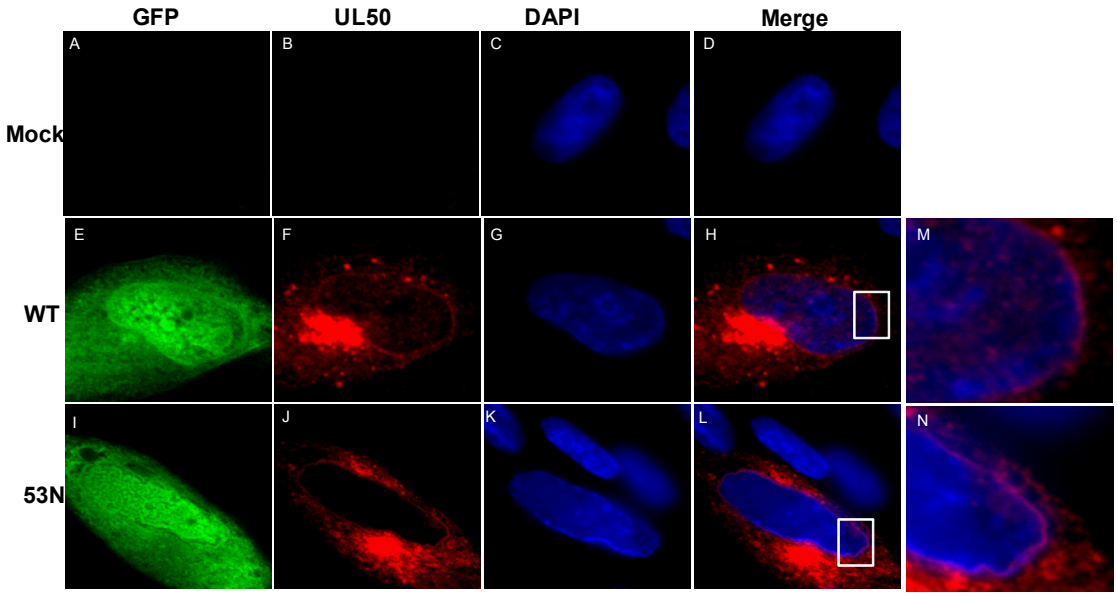
Supplemental Figure S2. Color version of Figure 2. CLEM analysis of HCMV UL50 and UL53 null mutants. HFF cells were electroporated with *UL50* null (50N, A-D), or *UL53* null (53N, E-H) pBADGFP or the rescued derivatives 50NR (I) or 53NR pBADGFP (J), and were seeded on gridded cover slips. Phase and fluorescence microscopy was performed on electroporated cells (panels A and E) on day 7 post electroporation and the cells were then processed for EM (panels B-D, F-J). The original GFP positive cells (panels A and E) were traced back and serial sections were scanned for nuclear and cytoplasmic nucleocapsids, for each sample. The insets in panels B,C and F,G indicate regions magnified in the next two panels (C,D and G,H, respectively). Bars represent 500nm. N, nucleus; cyt, cytoplasm. Arrows point to examples of nuclear A, B, and C capsids; cB and cC refer to cytoplasmic B and C capsids, respectively.



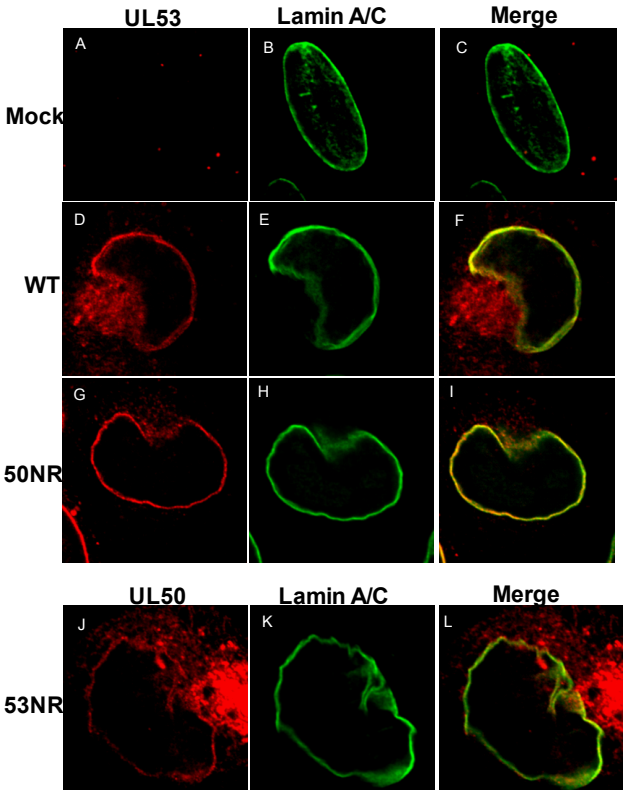
Supplemental Figure S3. Characterization of HCMV UL53-FLAG AD169-RV. A. Replication kinetics of the UL53-FLAG AD169-RV virus were compared with those of WT virus in a growth curve where viruses were applied at an MOI of 1 PFU/cell to 10^5 HFF/well in a 24-well cluster plate format. For each data point, titers were calculated by averaging plaque counts from duplicate titrations. Because counts from duplicate titrations differed less than twofold for all data points, error bars are not shown. B. HFFs grown on cover slips were mock-infected or infected with UL53-FLAGAD169-RV (53-F) at an MOI of 1. Cells were fixed at 72h p.i. and stained with antibody against FLAG (red) to visualize UL53-FLAG (panels i and iv) and the nucleus was stained with DAPI (blue, panels ii and vi). Images were acquired using confocal microscopy.



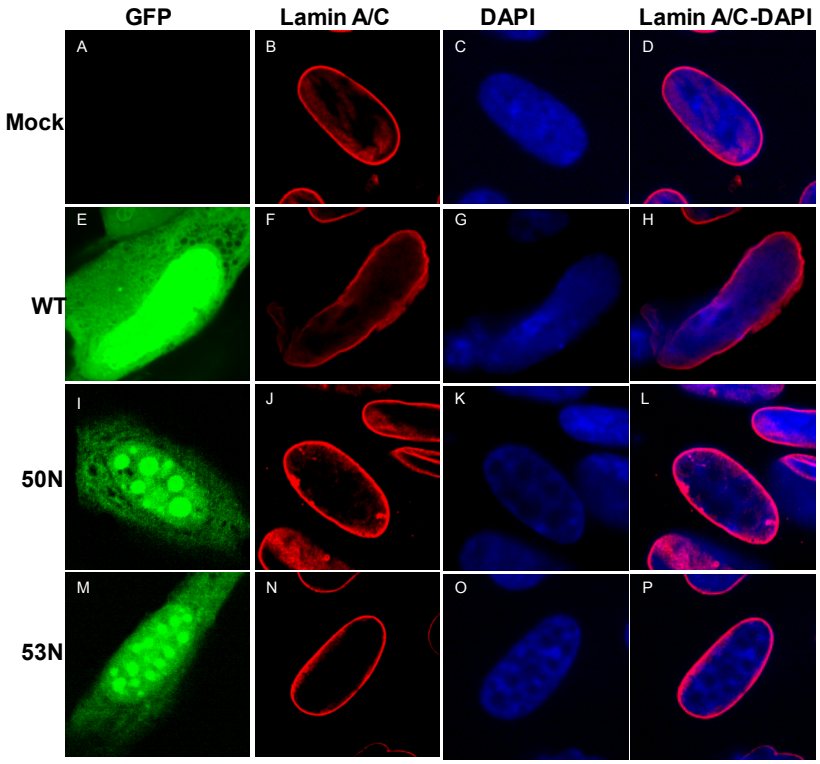
Supplemental Figure S4. Cellular distribution of HCMV UL53 in the absence of UL50. HFFs were mock electroporated (A-D) or electroporated with WT (E-H) or *UL50* null (50N, I-L) pBADGFP carrying FLAG-tagged UL53. Cells were fixed on day 7 and stained with anti-FLAG antibody (red) and DAPI (blue) and visualized by confocal microscopy, with GFP-positive cells identified by green fluorescence.



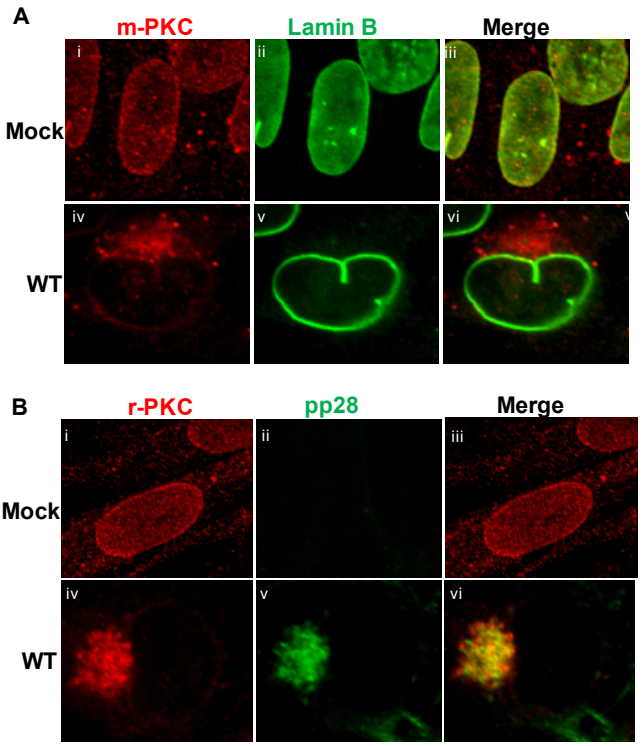
Supplemental Figure S5. Cellular distribution of HCMV UL50 in the absence of UL53. HFFs were mock electroporated (A-D) or electroporated with WT (E-H) or *UL53* null pBADGFP (53N, I-N). Cells were fixed on day 7 and stained with anti-UL53 antibody (red) or DAPI (blue) and visualized by confocal microscopy, with GFP-positive cells identified by green fluorescence. The insets indicate representative regions magnified 3X in panels M and N.



Supplemental Figure S6. UL50 and UL53 distribution patterns in infection with rescued derivatives. HFF cells were mock infected (A-C) or infected with WT (D-F), the rescued derivative of the *UL50* null mutant (50NR, G-I), or the rescued derivative of the *UL53* null mutant (53NR, J-L) at an MOI of 1. Cells were fixed at 72h p.i.. Samples were stained with antibodies against lamin A/C (green) and either UL53 (A-I) or UL50 (J-L) (both in red) and visualized using confocal microscopy.



Supplemental Figure S7. Color version of Figure 3. Nuclear lamina structure in the absence of HCMV UL50 or UL53. HFFs were mock-electroporated (A-D) or electroporated with WT (E-H), *UL50* null (50N, I-L) or *UL53* null (53N, M-P) pBADGFP. Cells were fixed on day 7 and stained with antibody against lamin A/C (panels B, F, J and N) and the nucleus was stained with DAPI (panels C, G, K and O). GFP positive cells (panels A, E, I and M) were visualized using confocal microscopy.



Supplemental Figure S8. PKC distribution in HCMV infected cells. A. HFF cells were mock infected (i-iii) or infected with WT HCMV (iv-vi), at an MOI of 1. Cells were fixed at 72h post infection. Samples were stained with mouse pan-PKC antibody (m-PKC, red) and lamin B (green) and visualized using confocal microscopy. B. HFF cells were mock infected (i-iii) or infected with WT HCMV (iv-vi), at an MOI of 1. Cells were fixed at 72h post infection. Samples were stained with rabbit pan-PKC antibody (r-PKC, red) and pp28 (green) and visualized using confocal microscopy.