

1 Supplementary Figs. S1-S4 for:  
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4 **Human cytomegalovirus mediates APOBEC3B relocalization early during infection**  
5 **through a ribonucleotide reductase-independent mechanism**  
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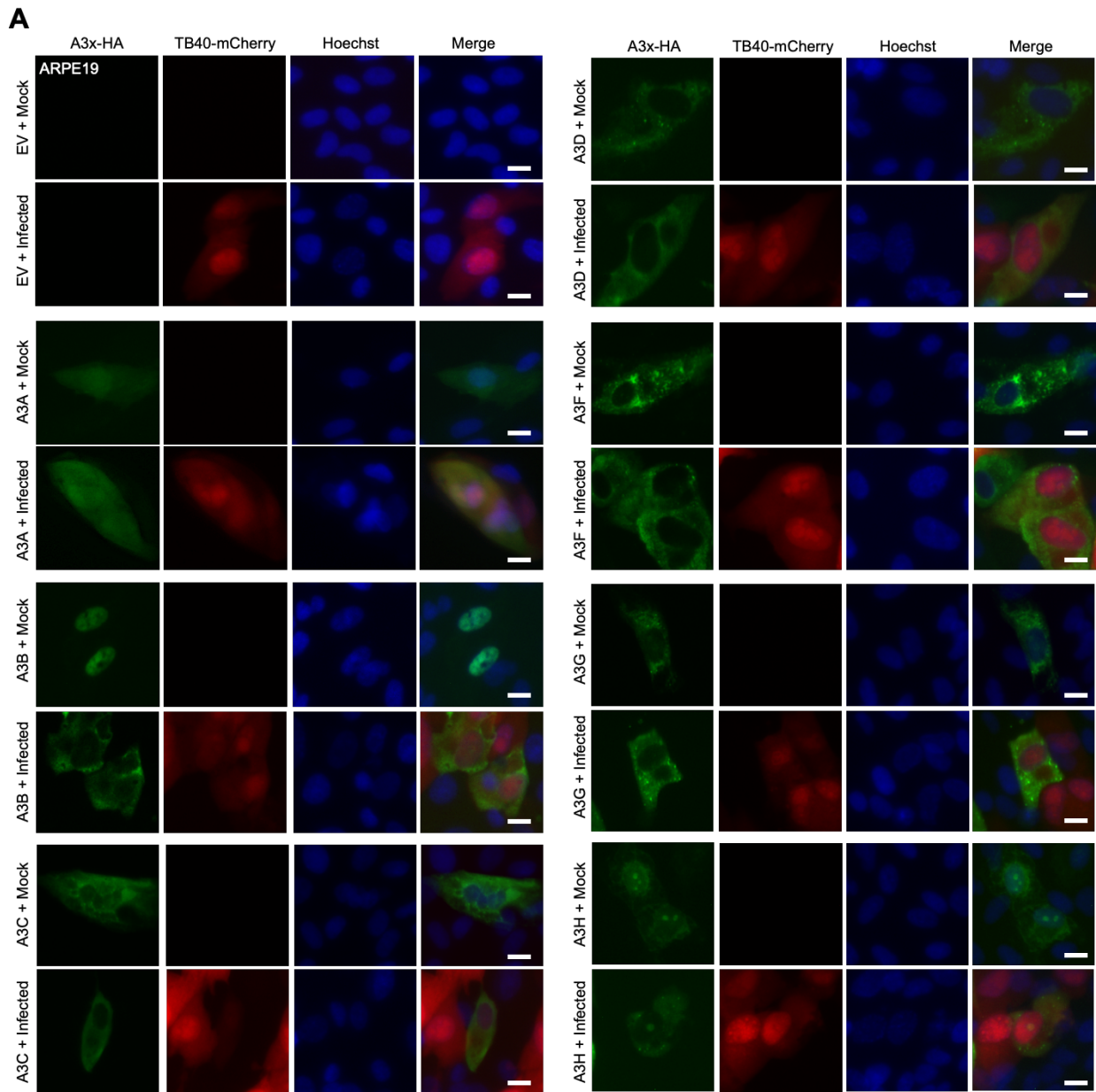
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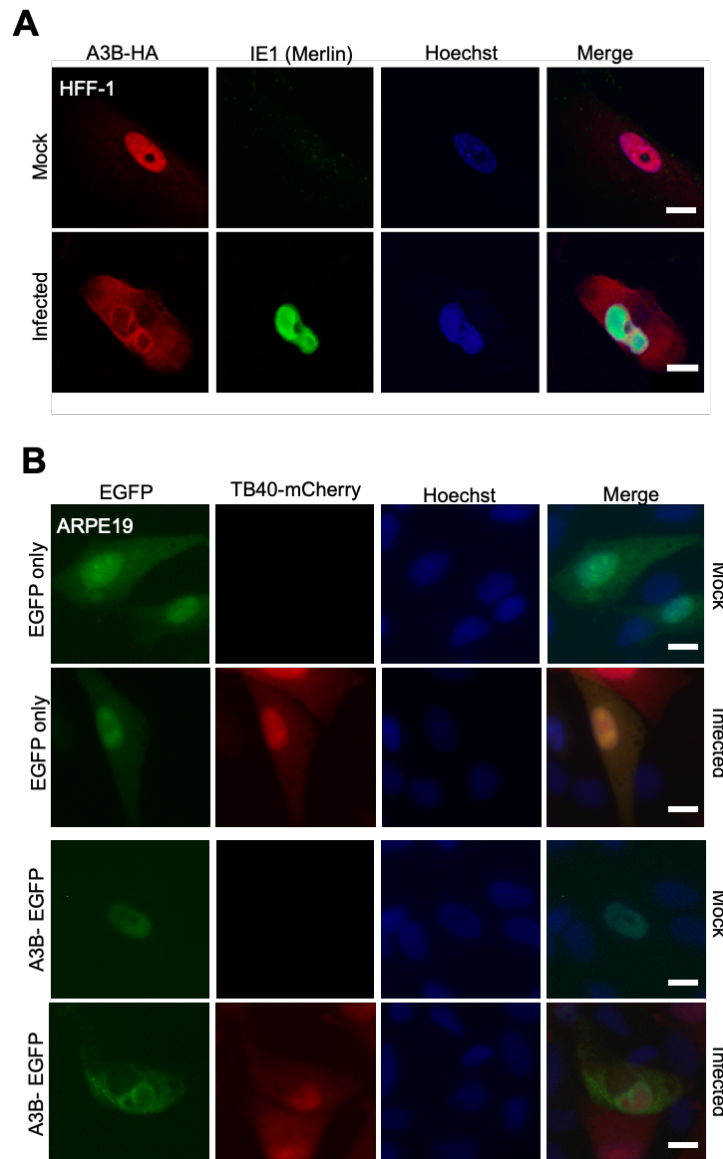
34 **Fig. S1. Human A3 subcellular localization phenotypes following HCMV infection.**

35 Representative IF microscopy images of ARPE19 cells transfected with the indicated

36 A3x-HA construct (green), infected with TB40-mCherry (red), and imaged 72 hpi (10  $\mu$ m

37 scale). All constructs encode wildtype human A3 proteins except A3A-E72A, which has

38 a catalytic mutation to prevent toxicity.



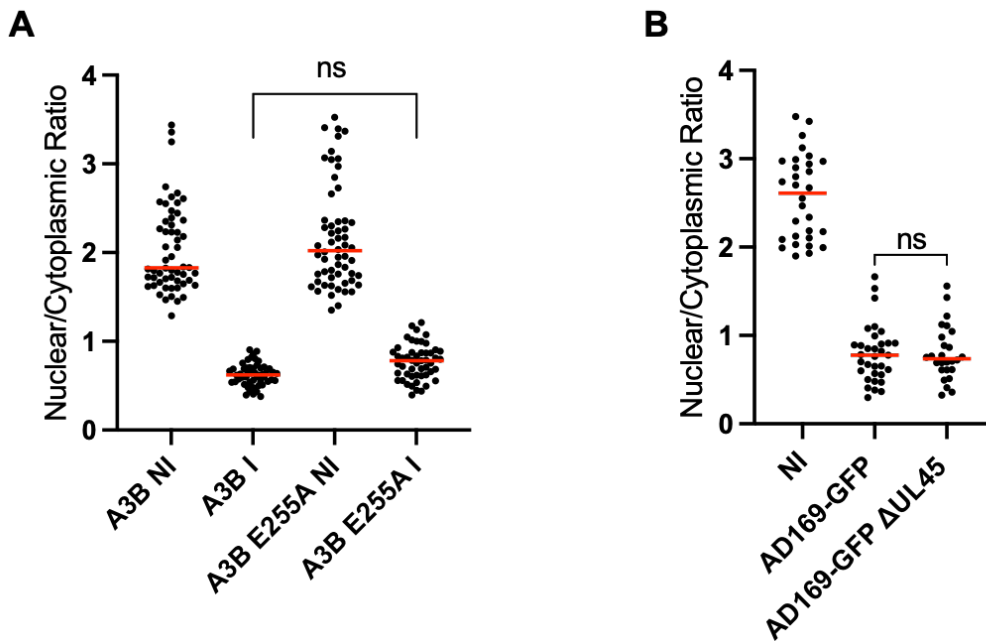
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**Fig. S2. A3B is relocated by HCMV Merlin and TB40-mCherry.**

44 (A) Representative IF microscopy images of HFF-1 cells expressing A3B-HA (red), mock  
45 or infected with Merlin strain (green) for 72 hrs (10  $\mu$ m scale).

46 (B) Representative IF images of ARPE19 cells transfected with EGFP only or A3B-EGFP  
47 (green), mock or infected with TB40-mCherry (red) for 72 hrs (10  $\mu$ m scale).

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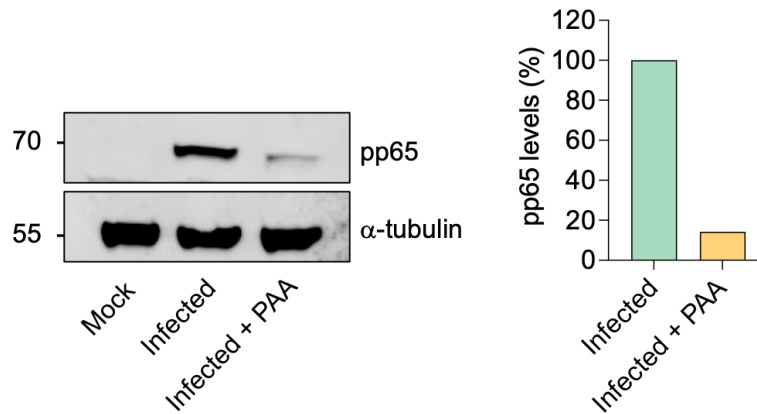
53 **Fig. S3. Quantification of A3B and A3B-E255A relocalization phenotypes.**

54 **(A)** HFF-1 stably expressing wt A3B or A3B-E255A were not infected (NI) or infected (I)  
55 with the AD169-GFP strain for 72 hrs. Quantification of Nuclear/Cytoplasmic Ratio of  
56 signal intensity for A3B or A3B-E255A with results showing that infection causes similarly  
57 strong relocalization phenotypes for both proteins (n>50 cells per condition; red lines are  
58 average values; ns, not significant, p = 0.34 by unpaired student's t-test).

59 **(B)** U373 stably expressing A3B or A3B-E255A were not infected (NI) or infected (I) with  
60 AD169 or AD169ΔUL45 for 72 hrs. Quantification of Nuclear/Cytoplasmic A3B Intensity  
61 Ratio, with both viruses triggering similar relocalization phenotypes (n>25 cells per  
62 condition; red lines are average values; ns, not significant, p = 0.94 by unpaired student's  
63 t-test).

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67 **Fig. S4. PAA treatment blocks late protein accumulation.**

68 Infected cells were treated or not with PAA for 48 hrs and then immunoblotting was used  
69 to evaluate expression levels of the late HCMV gene product pp65 (left, with band  
70 quantification to the right).

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