# Supporting Information

**Utley *et al.* 10.1073/pnas.0712144105**

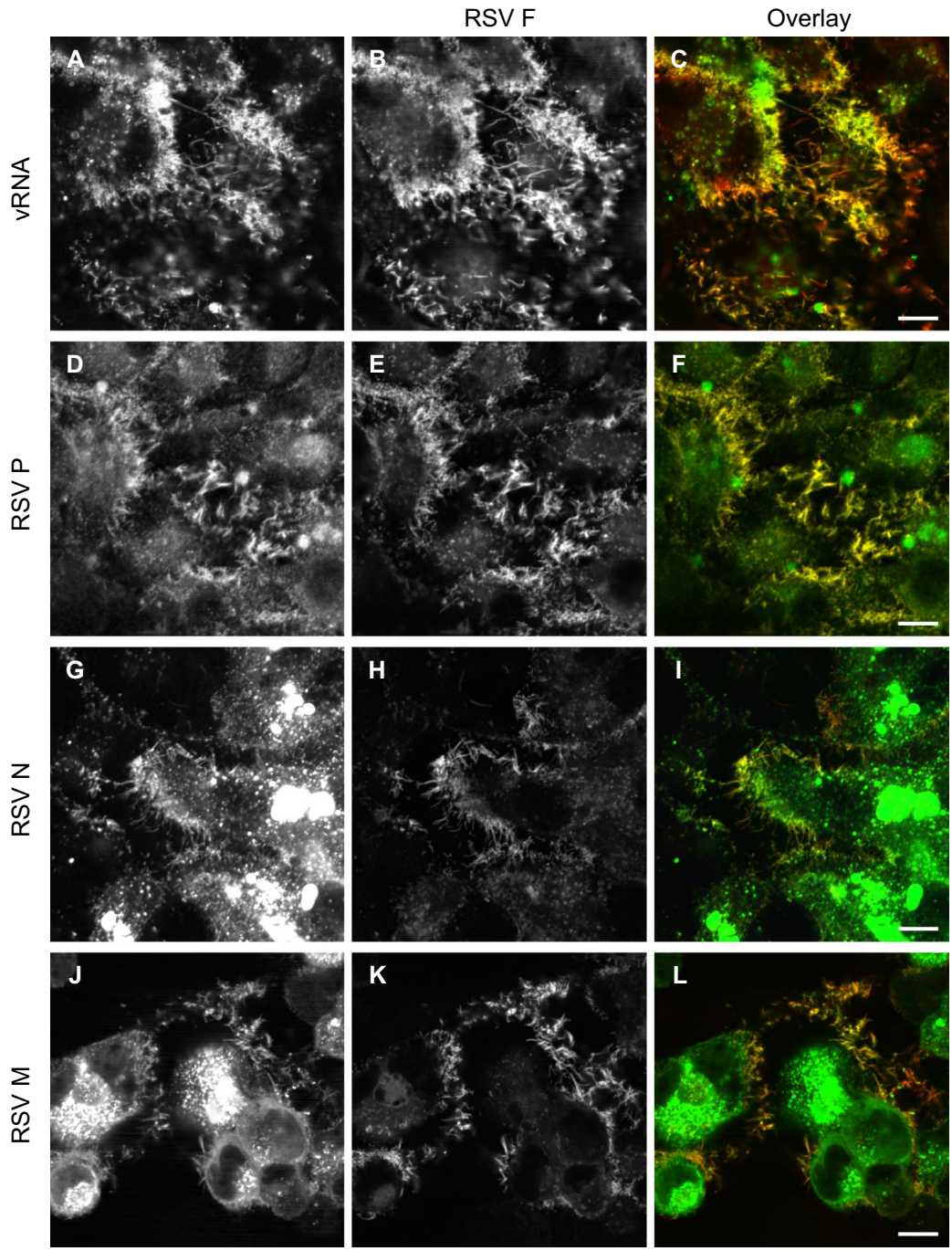


**Fig. S1.** Viral filaments contain all of the necessary RSV structural proteins. (*A*, *D*, *G*, and *J*) FIP2-ΔC2 cells infected with RSV for 3 days were assayed for the presence of genomic vRNA with molecular beacons (*A*) or by indirect immunofluorescence for the major structural proteins P, N, and M (*D*, *G*, and *J*, respectively). (*B*, *C*, *E*, *F*, *H*, *I*, *K*, and *L*) Localization of surface filaments is demonstrated by indirect immunofluorescence of the RSV F protein, which decorates surface filaments (*B*, *E*, *H*, and *K*). Genomic vRNA, viral structural proteins P, N, or M are pseudocolored green, and RSV F glycoprotein is pseudocolored red in the merged images (*C*, *F*, *I*, and *L*, respectively).

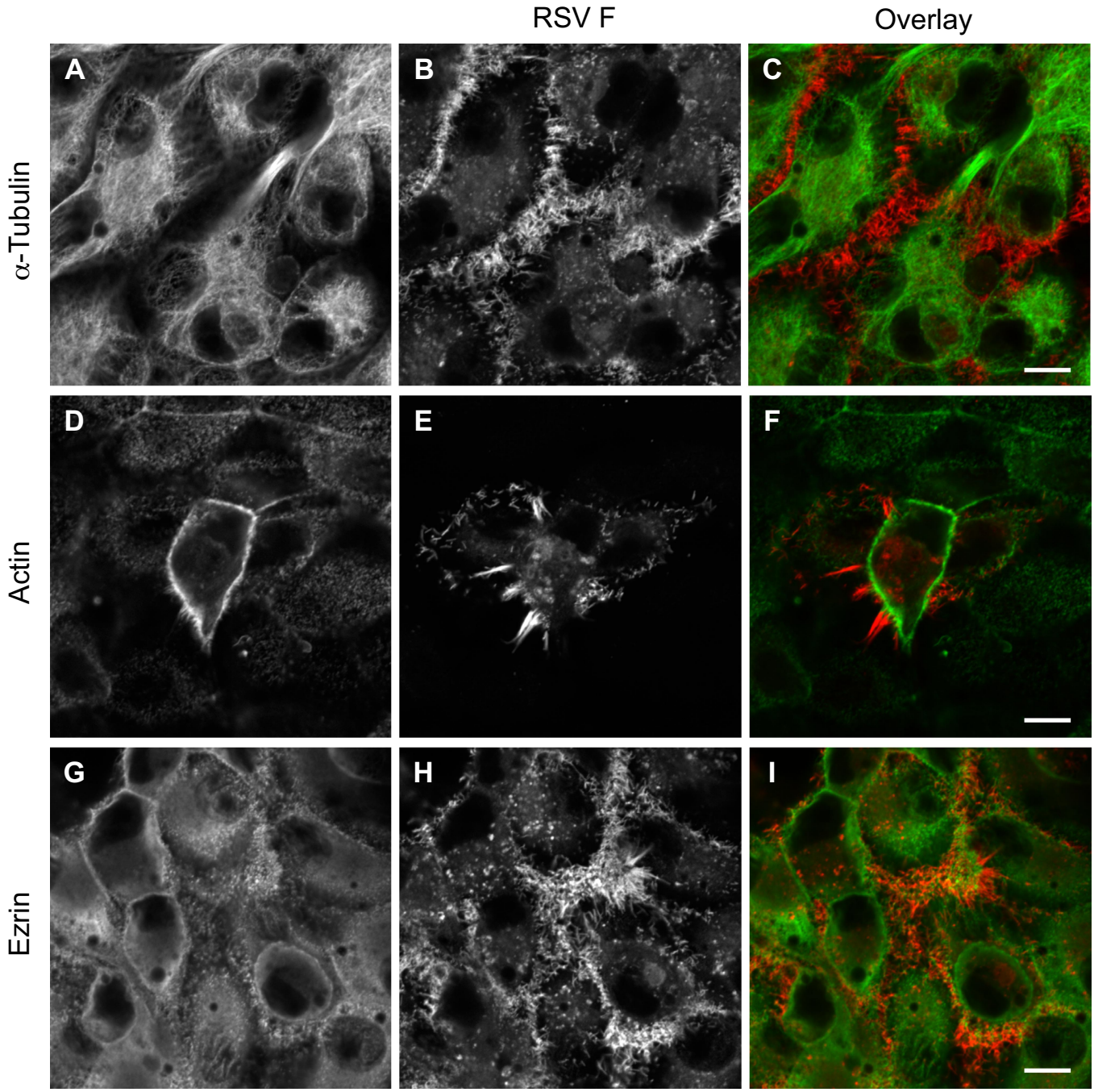


**Fig. S2.** Virus-induced filaments do not contain major cytoskeletal elements. (*A*, *D*, and *G*) FIP2-ΔC2 cells inoculated with RSV were assayed 3 days later for the presence of microtubules, actin, or ezrin, respectively, in surface filaments by fluorescence microscopy. (*B*, *E*, and *H*) Localization of surface filaments is demonstrated by indirect immunofluorescence detection of RSV F protein. (*C*, *F*, and *I*) Cytoskeletal elements are pseudocolored green, and RSV F glycoprotein is pseudocolored red in the merged images.

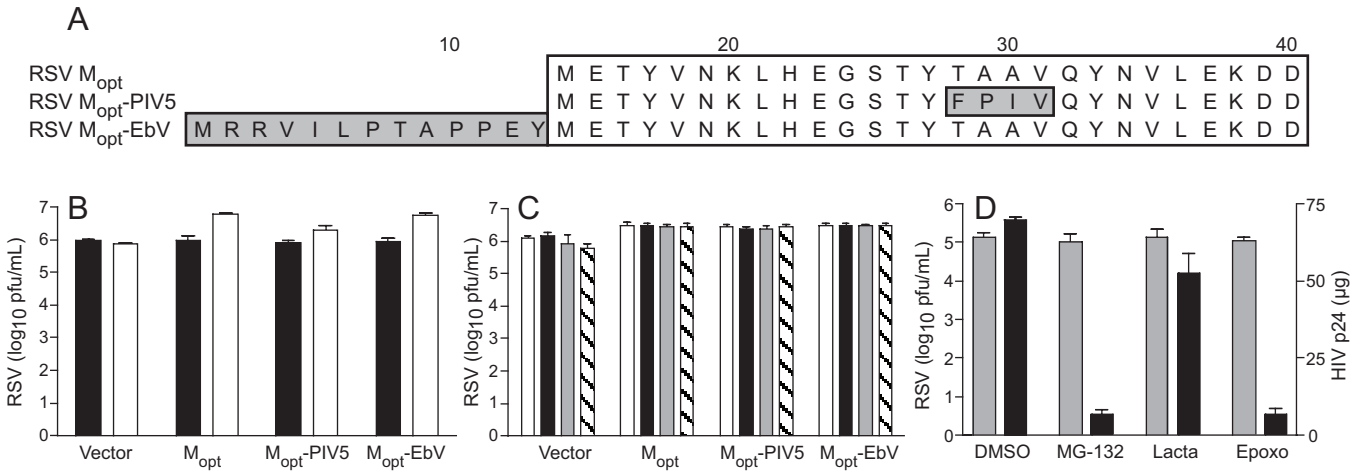


**Fig. S3.** Neither proteasome inhibitors nor insertion of a strong late domain alter RSV budding. (*A*) RSV matrix protein variant sequences are aligned to show the N-terminal 40 aa of optimized RSV matrix ($M_{opt}$) and location of the engineered insertion of the FPIV late domain from PIV5 ($M_{opt}$-PIV5) or the N-terminal 15 aa including the PTAPPEY late domain of Ebola virus ($M_{opt}$-EbV). (*B*) HEp-2 cells transfected with M protein variants were inoculated with RSV at a moi of 0.25. Cell-associated titer (filled squares) and supernatant virus titer (open squares) were determined by plaque assay on day 5. (*C*) HEp-2 cells were cotransfected with DNA encoding a RSV M protein variant and EGFP (white bars), Vps4a-EGFP (black bars), Vps4a-K173Q (gray bars), or Vps4a-E228Q (striped bars). Cells were inoculated 24 h later with RSV at a moi of 0.25. Supernatant virus titer was determined by plaque assay on day 5. Transfection stability of Vps4 constructs was checked before collection by epifluorescence; expression was confirmed in all cases. RSV M proteins were tested separately for transfection stability (data not shown). (*D*) To determine the effect of proteasome inhibitors, HEp-2 cells were infected with RSV or $HIV_{NL4-3}$ at a moi of 2.0 and then incubated with either DMSO vehicle or the inhibitors epoxomicin (Epoxo), lactacystin (Lacta), or MG-132. RSV supernatant plaque titer (gray bars) or HIV supernatant p24 (black bars) were determined at 48 h. Data shown are mean ± SD.