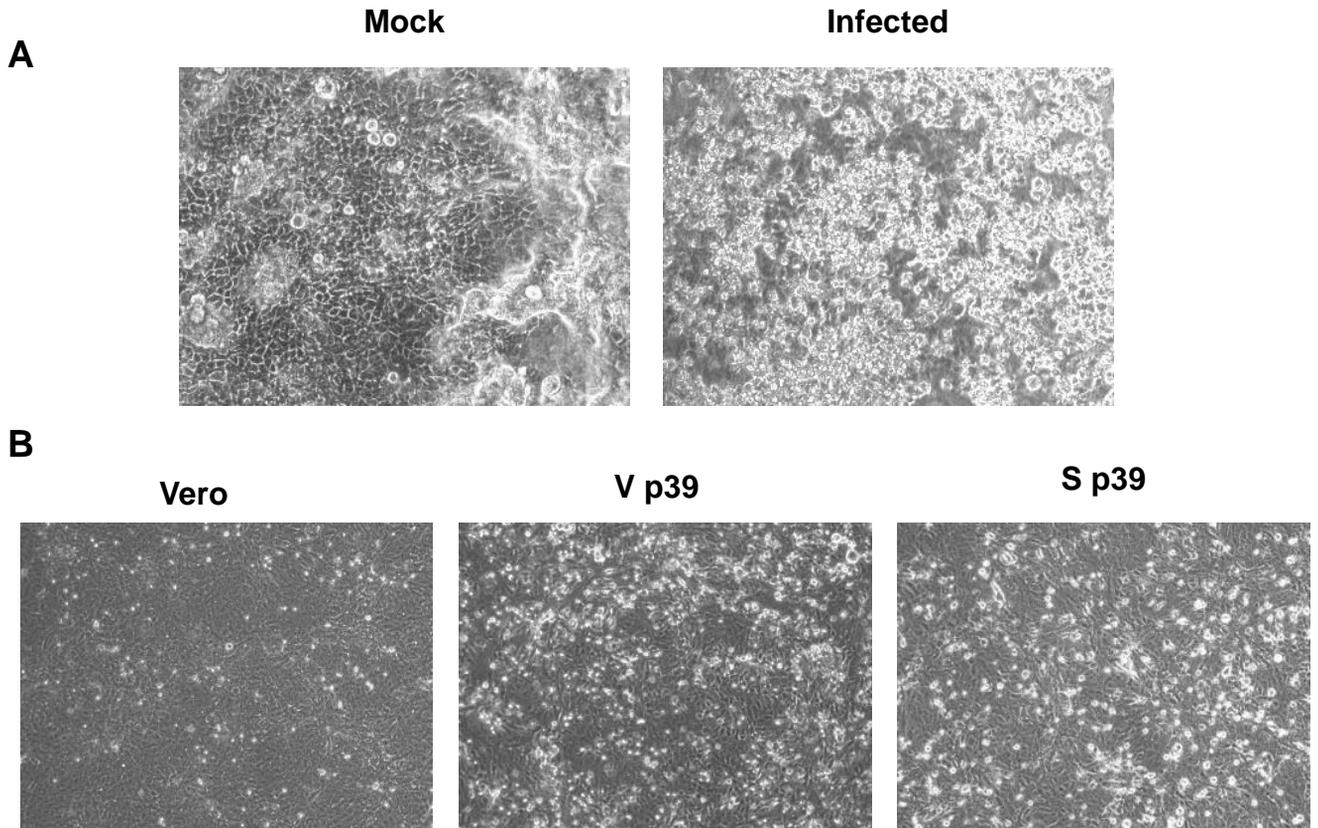
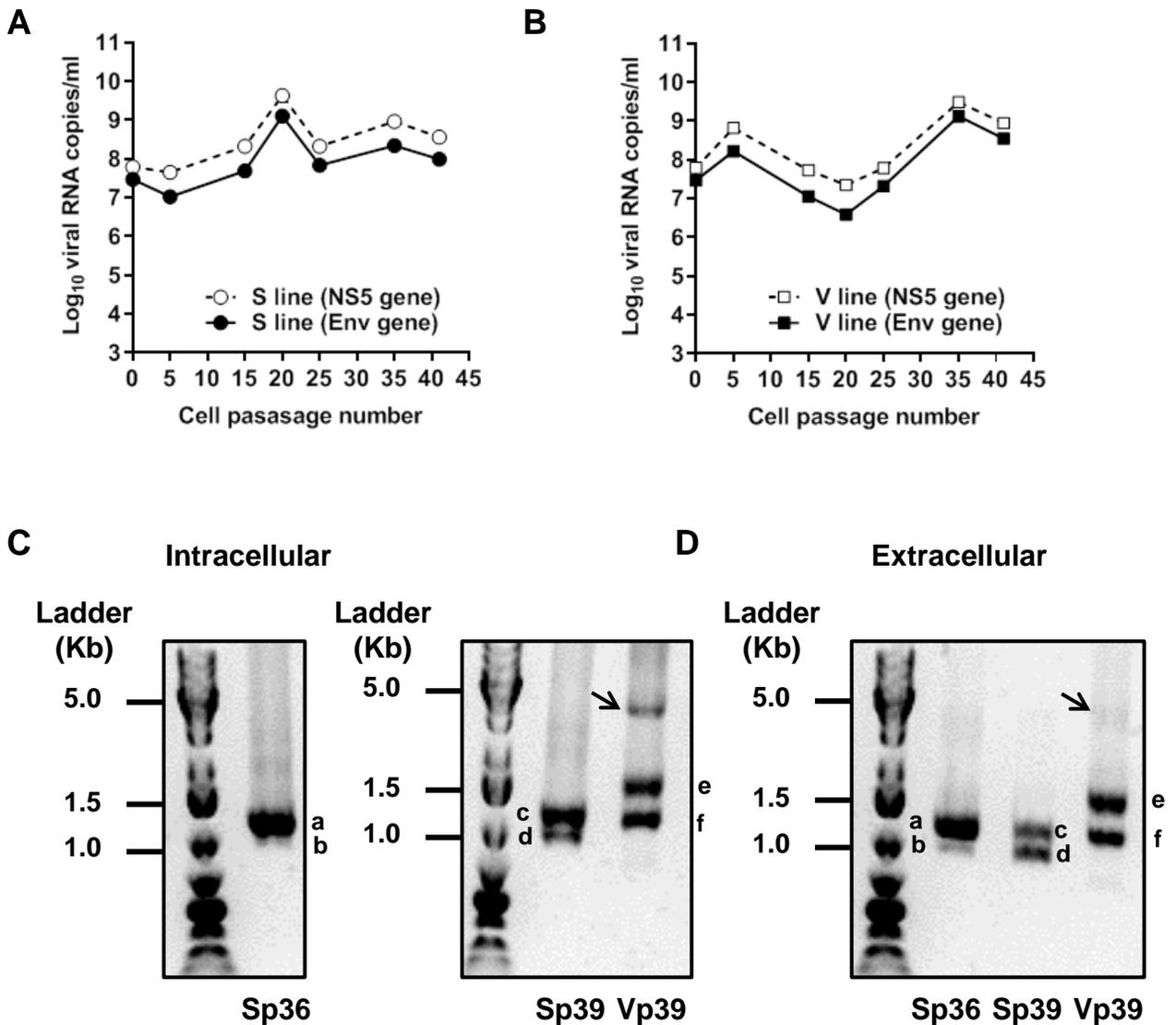


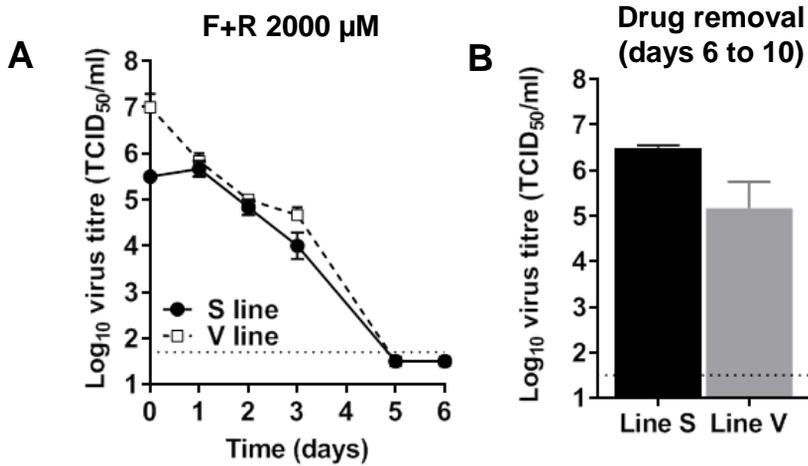
## Establishment of a cell culture model of persistent flaviviral infection: Usutu virus shows sustained replication during passages and resistance to extinction by antiviral nucleosides



**Figure S1.** Cellular monolayers of Vero cells infected with USUV. A, Lytic infection (right) compared to Mock-infected cells (left) in 96-well plates. Mock cells show multilayer formations (right). B, Monolayers of uninfected Vero, V p39 and S p39 at day 5 after seeding in 75 cm<sup>2</sup> flasks.



**Figure S2.** Viral RNA in cell supernatants along passages. The number of molecules was determined by qPCR, using primers for the specific detection of Env- (black symbols) or NS5-coding regions (white symbols). C-D, Amplicons identified in samples obtained from persistently-infected cells V (passage 39, V p39) and S (passages 36 and 39, S p36 and S p39) using primers spanning residues 1 to 3359. C, PCR amplification of viral RNA extracted from cells. D, PCR amplification of viral RNA extracted from cellular supernatants after micrococcal nuclease treatment to eliminate non-encapsidated genomes, as previously described (Arias et al 2014 eLife; ;3:e03679). Different amplicon lengths detected in V p39 and S p39 intracellular and extracellular RNA extracts are indicated with a lowercase letter (a to f). An arrow indicate the detection of full-length genome amplicons.



**Figure S3.** Prolonged exposure of persistently-infected cells to a cocktail of FAV and RBV eliminates viral infectivity in the supernatant. A, Continuous treatment of V and S cells at passage 34 with a combination of FAV and RBV (F+R) at a concentration of 2000  $\mu$ M each, leads to undetectable levels of infectious virus. Cells were treated with F+R during 6 days. At day 3, cellular supernatants were removed and fresh media containing F+R was added to the cells. B, At day 6, drugs were removed and the cells cultured for 4 additional days. Removal of drug treatment leads to a relapse in virus titres in the supernatant of both V and S cells.