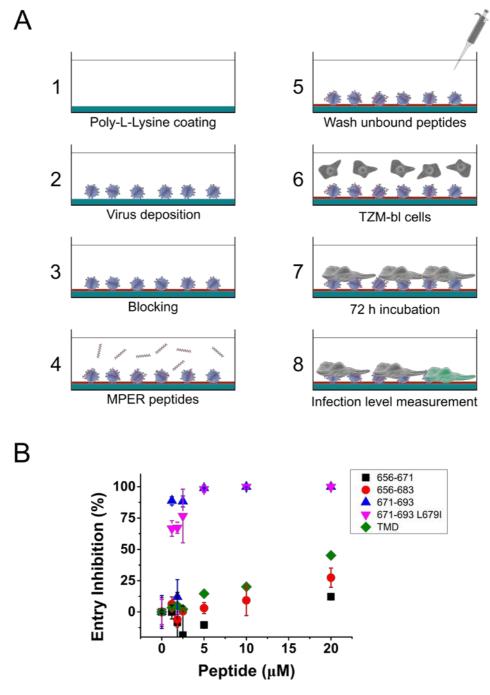
## **Supplemental Information**

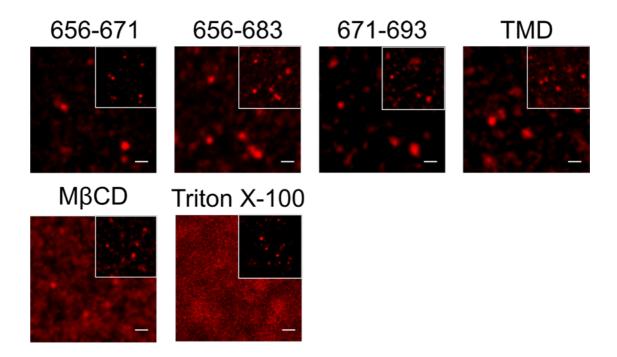
## Effects of HIV-1 gp41-Derived Virucidal Peptides on Virus-like Lipid

## **Membranes**

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**Supporting Figure 1. Cell infection inhibition exerted by MPER-TMD-based peptides. A) Experimental set-up.** To measure cell entry inhibition, HIV-1 pseudoviruses were first pre-attached to lysine-coated plates (1-3). The immobilized particles were subsequently treated with increasing concentrations of the peptides (4). After washing (5), reporter TZM-bl cells were layered on top (6-7), and infectivity inferred from the number of total cells expressing GFP (8). **B) Control for sequence specificity.** In these assays VSV-G pseudoviruses were used as a control for cell entry mediated by a different viral fusion glycoprotein. Conditions otherwise as in Fig. 1B.



Supporting Figure 2. Fluorescence imaging of membrane lipids on solid supports containing immobilized viral particles. For fluorescent labeling of membrane lipids, transfected 293T cells were incubated with DiD probe prior to isolation of the pseudoviruses as described in: Padilla-Parra et al. (2013) PLOS ONE, e71002. Particles were attached to a poly-Lys-coated surface for imaging. Micrographs on top illustrate the effect of the different peptides. Images were taken after 15 min incubation with peptides applied at 20  $\mu$ M, a concentration that results in full infection inhibition in the case of MPER(671-693) (see Fig. 1B). Micrographs below display samples treated with Methyl- $\beta$ -cyclodextrin (10 mM) or Triton X-100 (0.5%). Insets display micrographs of the samples prior to treatment with the different compounds. Scale bar is 1  $\mu$ m.