Supporting Information

Pharmaceutically acceptable carboxylic acid-terminated polymers show activity and selectivity against HSV-1 and HSV-2 and synergy with antiviral drugs

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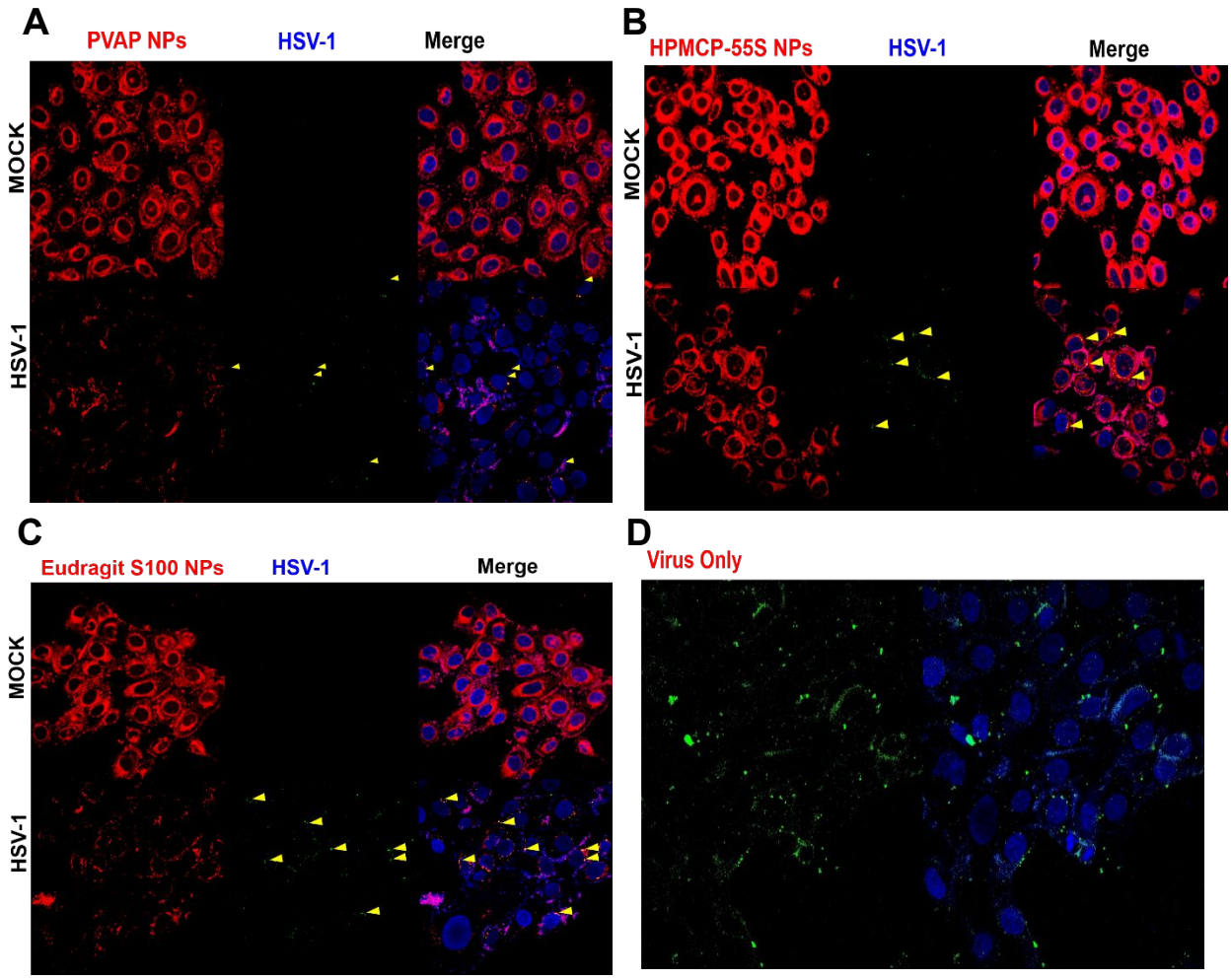

Figure S1: Understanding the interaction between fluorescent polycarboxylate nanoparticles and fluorescent HSV-1 in the presence of HCE cells. Polycarboxylate nanoparticles were labelled with fluorophore (Rhodamine-6G). HCE cells were seeded in an 8-chamber confocal microscopy slide (Nunc Lab-Tek, Thermo Fisher Scientific, Waltham, MA, USA) with 200 µL of complete MEM media. At 24 h post seeding, 10 µL of Rhodamine-6G labeled (A) PVAP (B) Eudragit S100 or (C) HPMCP-55S nanoparticles were either mixed with K26-GFP HSV-1 virus or mock control before they were added into each chamber of the 8-chamber plate. (D) Cells were also treated with only K26-GFP HSV-1 virus. After 2 h incubation, cells were fixed using 4% paraformaldehyde for 10 minutes. Nuc-blue™ (Thermo Fisher Scientific) DAPI stain was added to each well (2 drops per mL of solution) for 5 min before the images were procured using a confocal microscope. Images were taken at 488-505 nm for GFP virus and 525-581 nm for Rhodamine-6G. The cytoplasm of HCE cells treated with Rhodamine-6G labeled polycarboxylate nanoparticles (mock control) showed bright red fluorescent indicating extensive uptake by HCE cells. The HCE cells treated with Rhodamine-6G labelled nanoparticles and K26-GFP HSV-1 significantly lesser intracellular fluorescence indicating interaction between polymeric nanoparticles and HSV-1. The yellow arrows indicate the presence of neutralized virus particles present inside the cell.

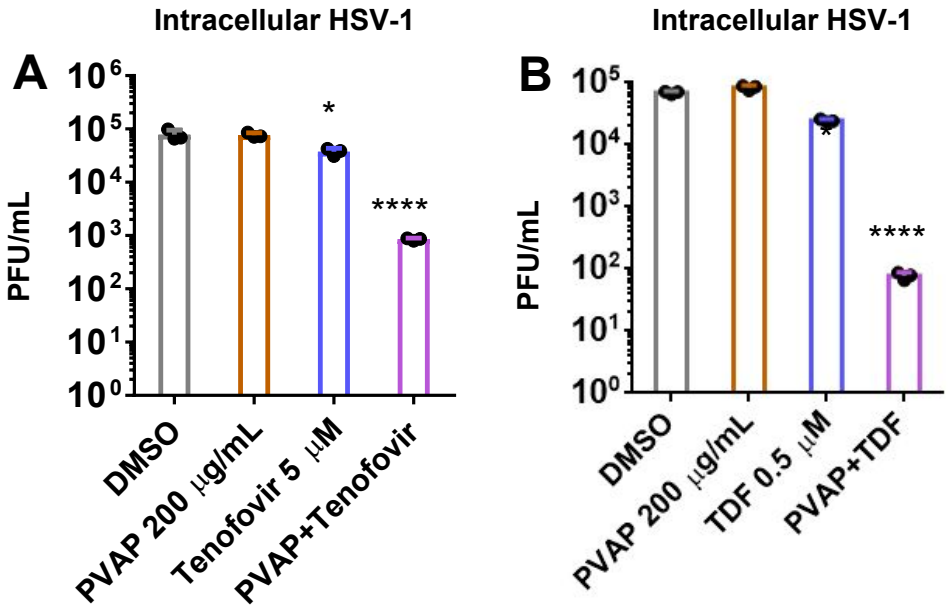

Figure S2: PVAP nanoparticles and tenofovir or tenofovir disoproxil fumarate show synergistic therapeutic activity against HSV-1 in vitro. HCEs were infected with 0.1 MOI HSV01 17 GFP and then therapeutically treated with either 200 µg/mL PVAP nanoparticles and Tenofovir (5 µM) individually or in combination, (A) Whole cell lysates were titrated for viral load using plaque assays. (B) In a similar experiment 200 µg/mL PVAP nanoparticles was synergized with TDF (0.625 µM) and tested on HSV-1 infected HCE cells. Asterisks indicate significance by multiple Students T-test : *p<0.05, ****p<0.0001.

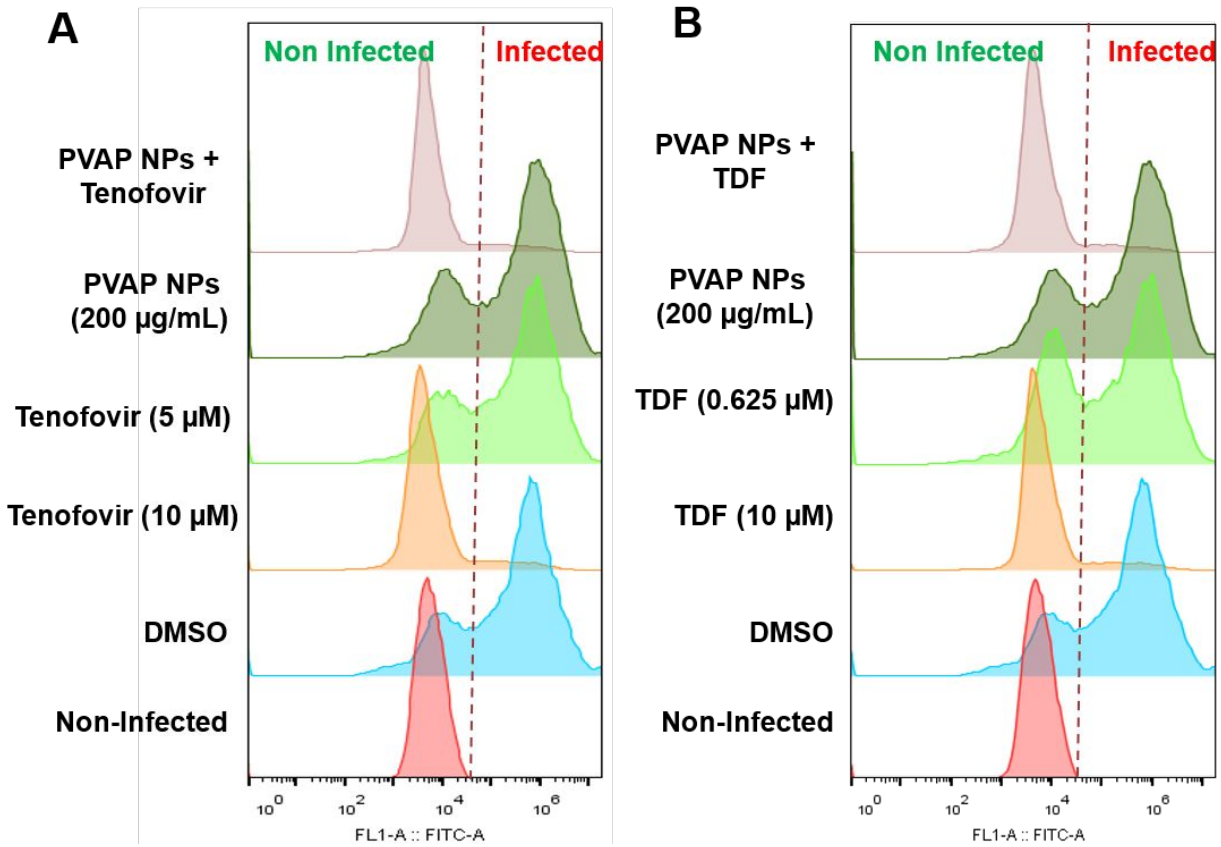

Figure S3: PVAP nanoparticles (NPs) and tenofovir or tenofovir disoproxil fumarate (TDF) show synergistic therapeutic activity against HSV-2 in vitro. (A) HeLa cells were infected with HSV-2 333 GFP virus at 0.1 MOI. At 2 hpi, cells were washed with PBS once and fresh media containing an ineffective concentration of PVAP NPs (200 µg/mL), ineffective concentration of tenofovir (5 µM), effective concentration of tenofovir (10 µM) and combination of ineffective concentration of PVAP NPs (200 µg/mL) and tenofovir (5 µM). The efficacy was evaluated qualitatively using flow cytometry analysis and the data was compiled using FlowJo Version 10.0. A no-infection, no-treatment control was used as fluorescence baseline for the experiments. (B) A similar experiment was performed using TDF instead of tenofovir at shown concentrations.