Supporting Information

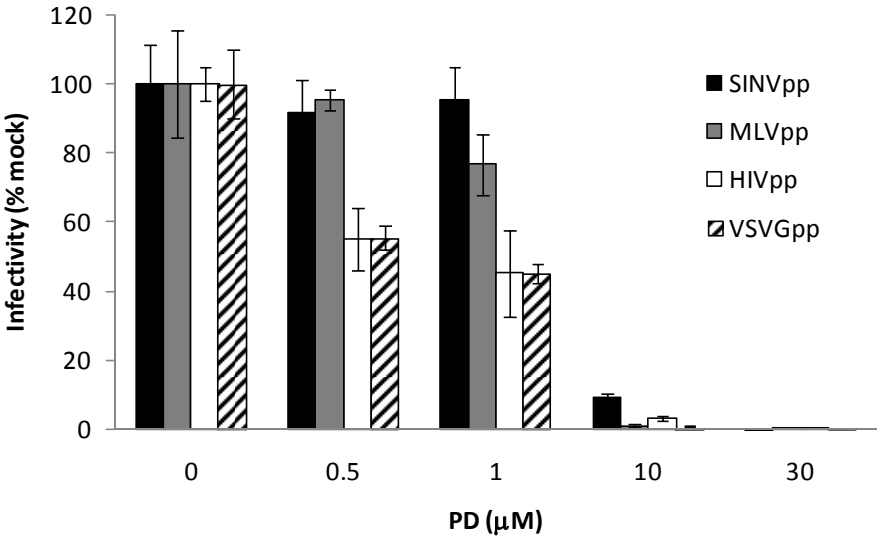

Fig. S1. PD inhibits infection by different pseudotyped lentiviruses. Lentiviruses (harboring pV1-Gluc provirus) pseudotyped with envelope proteins from Sindbis virus (SINVpp), murine leukemia virus (MLVpp), human immunodeficiency virus (HIVpp) and vesicular stomatitis virus (VSV-Gpp) were incubated with PD at 37°C for 30 min, and used to spinoculate BHK-J (SINVpp), Huh-7.5 (MLVpp, VSV-Gpp) and TZM-bl (HIVpp) cells at 4°C as described in *Materials and Methods*. Cells were washed 4 times with fresh medium to remove any unbound viruses and compound, and incubated at 37°C/5% CO_2. Viral infectivity was determined by measuring the supernatant activity of the Gluc reporter 48 h later. Due to differences in specific infectivity, SINVpp, MLVpp, HIVpp and VSV-Gpp virus stocks were diluted 5-, 50-, 10- and 100-fold, respectively, with fresh complete growth medium prior to compound treatment. The different virus dilutions ensured a similar final titer of the different viruses, as judged by the similar supernatant activities of the Gluc reporter after dilution. The error bars represent the mean ± SD of 2 independent experiments performed in duplicate.

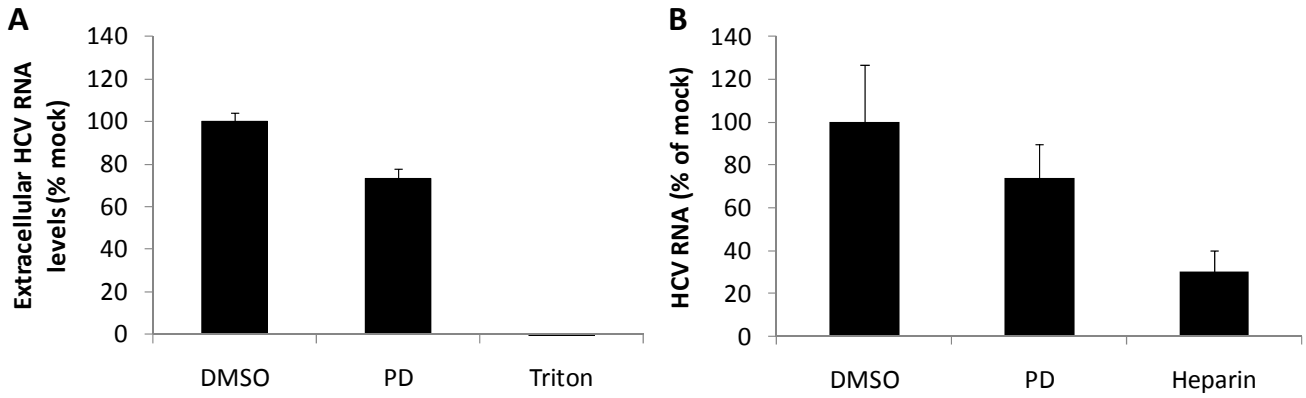

Fig. S2. Effect of PD on HCVcc virion integrity and attachment to cells. (**A**) **PD only weakly disrupts HCVcc.** HCVcc (10^4 $TCID_{50}$/mL) was incubated with PD (300 μM), Triton X-100 (0.1%) or 1% DMSO in the presence of 7 ng/mL RNase A at 37°C for 90 min. Isolation and quantification of viral RNA was carried out as described in *Material and Methods*. (**B**) **HCVcc cell attachment assay.** Jc1 HCVcc was partially clarified by four serial passages through a 300 kDa cutoff ultrafiltration membrane (Pall Life Sciences, Port Washington, NY). With each passage through the centrifugal unit, the retained virus was diluted in PBS prior to the next passage. HCVcc (10^4 $TCID_{50}$/ml) was pre-incubated with either freshly prepared heparin (1000 μg/ml; positive attachment inhibitor control) from porcine intestinal mucosa (Sigma, St. Louis, MO), 300 μM PD, or 1% DMSO at 37°C for 90 minutes under low-serum (<1%) conditions. In preparation for virus addition, Huh-7.5 cells seeded one day earlier at $3x10^5$ cells/well in 24-well plates were chilled on ice for 5 minutes. After aspirating the existing medium from the cells, 50 μl of the pre-treated HCVcc was added per well and the virus/cell mixture was incubated at 4°C for an additional 3 h. Cells were subsequently washed 5 times with complete growth medium and incubated at 37°C/5% CO_2 for an additional 2 h. Total RNA was harvested from the cells using the RNeasy Mini Kit (Qiagen, Valencia, CA). RNA levels of cell-bound HCVcc were determined via TaqMan qRT-PCR as described in *Materials and Methods*. Note: The observed slight decrease in attachment of PD-treated HCVcc relative to DMSO-treated virus is likely due to virion lysis (see (A)). The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

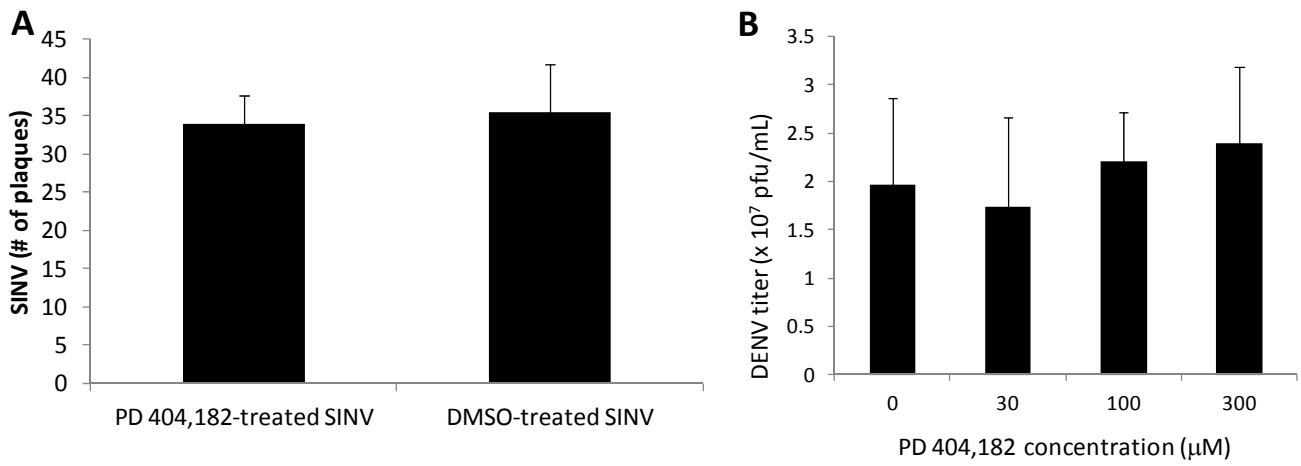

Fig. S3. Effect of PD on SINV and DenV. (**A)** Sindbis virus was produced in cell culture by electroporation of BHK-J cells with *in vitro*-transcribed viral RNA. Briefly, plasmid carrying the genome of SINV (Toto1101) (2) was linearized by digestion with *Xho*I and 1 μg of the linearized plasmid was used as a template for run-off transcription with SP6 RNA polymerase (Ampliscribe SP6 High-Yield Transcription Kit, Epicentre, Madison, WI). BHK-J cells were trypsinized, resuspended in cold DPBS to $2.8x10^7$ cells/ml and 400 μl of this cell suspension was electroporated with 3 μg of *in vitro*-transcribed viral RNA using an ECM 830 electroporator (Harvard Apparatus, Holliston, MA) using the following settings: 750 V, 5 pulses, 99 us pulse length, 1 second intervals. Virus-containing supernatant was collected 24 h post electroporation and stored at -80°C. Virus titer was determined on BHK-J cells with 10-fold serial dilutions of sample, and then plaques were visually enumerated after crystal violet staining, as previously described (3). For determination of the inhibitory effect of PD 404,182, cell culture-produced SINV was diluted 1000-fold in complete growth medium to 10^5 pfu/ml and pre-incubated with 300 μM PD 404,182 or 1% DMSO at 37°C for 1 h. Pre-incubated virus was diluted a further 2000-fold and used to inoculate BHK-J cells for enumeration of plaques. (**B**) Serotype 2 New Guinea C strain Dengue virus was propagated in Vero cells. Dengue virus serially diluted in complete medium containing 10% FBS was incubated with PD (10, 100 or 300 μM) or DMSO at 37°C for 30 min and used to infect Vero cells in a standard plaque assay. Briefly, Vero cells were seeded in 24-well plates at 10^5 cells/well and inoculated with 100 μL PD- or mock-treated Dengue virus at 37°C for 1 h. After removal of the inoculum, these cells were overlayed with 1 ml of culture medium containing 0.5% methyl cellulous. Five days later, the cells were fixed and stained with crystal violet to visualize plaques. The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

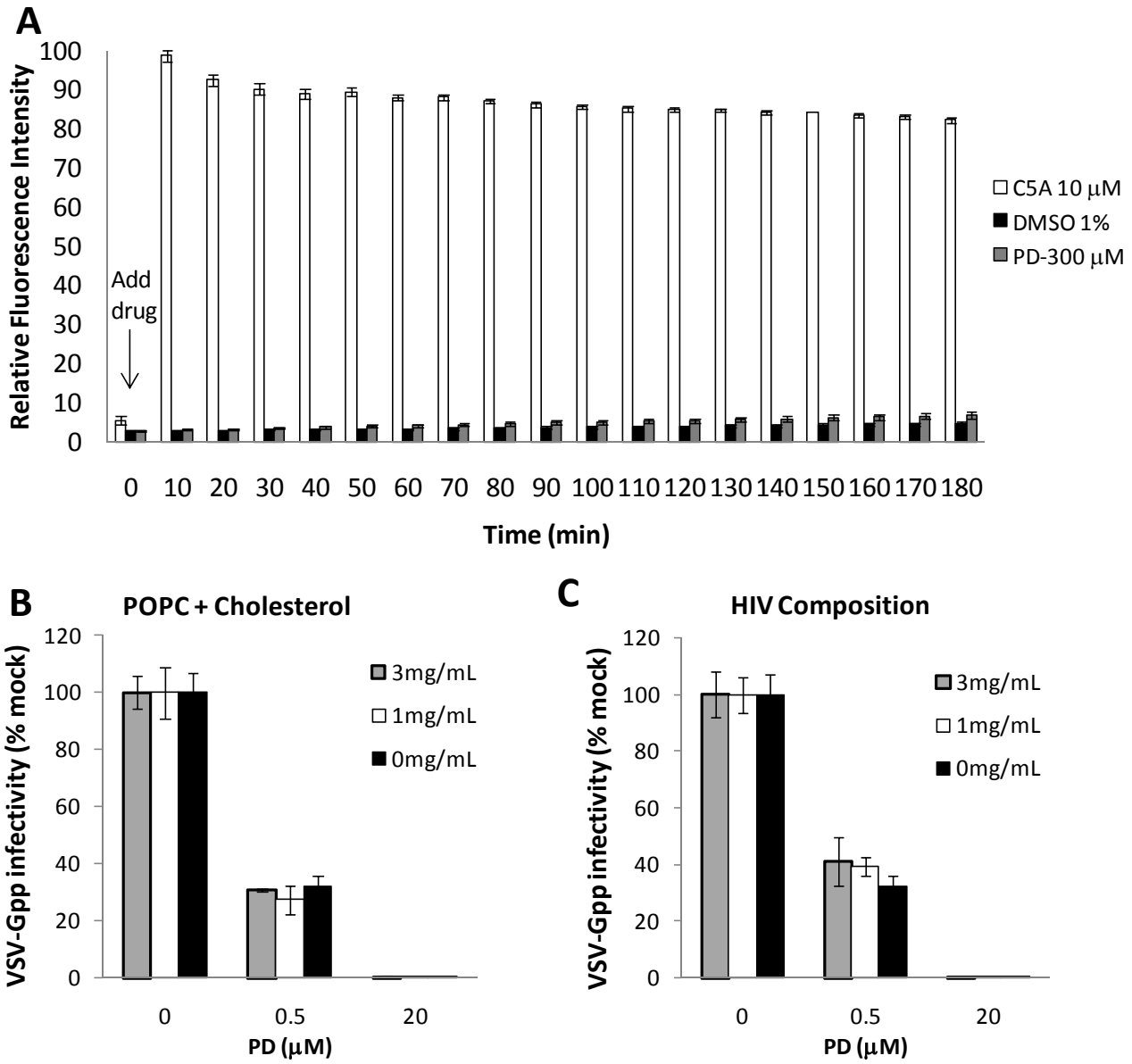

Fig. S4. (**A**) No significant increase in fluorescence intensity was observed even after prolonged incubation of liposome with PD. (**B, C**) The virocidal activity of PD is not attenuated by the presence of liposomes. Liposomes composed of (B) 70 mg POPC and 30 mg cholesterol or (C) 12 mg POPC, 33 mg SM, 5 mg PE, 19 mg pl-PE, 30 mg cholesterol and 1 mg POPS (the same composition as HIV (4)) per 100 mg were incubated with PD and VSV-Gpp as described in Fig. 3B. The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

POPC: 1-Palmitoyl-2-Oleoyl-sn-Glycero-3-Phosphocholine; Cho: cholesterol; SM: sphingomyelin; PE: 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylethanolamine; POPS: 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine; pl-PE: 1-alkenyl,2-acylglycerophosphoethanolamine (Avanti Polar Lipids, Inc., Alabaster, AL); DPPC: 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) (Fisher Scientific, Pittsburg PA).

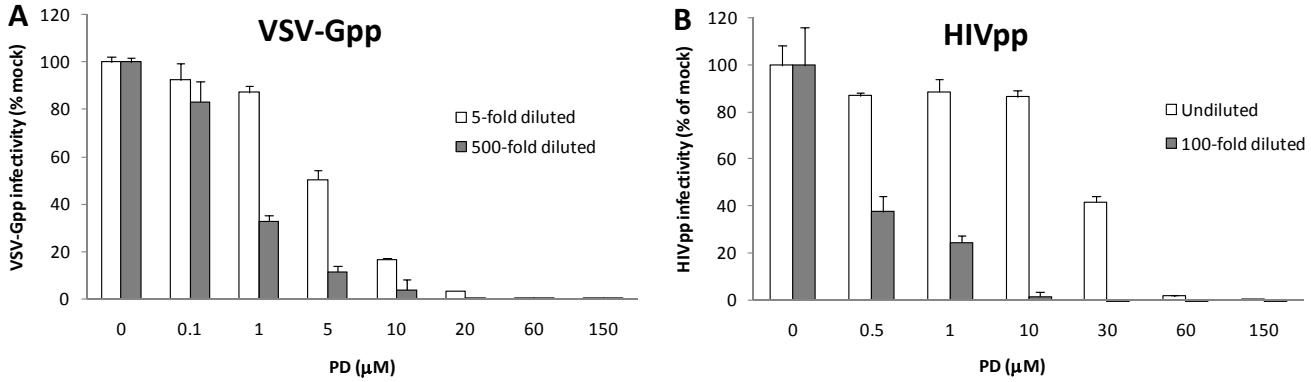

Fig. S5. The antiviral potency of PD against VSV-Gpp and HIVpp is virus dilution-dependent. VSV-Gpp (**A**) and HIVpp (**B**) (both harboring pV1-Gluc provirus) diluted in fresh complete growth medium and treated with PD at 37°C for 30 min were used to spinoculate Huh-7.5 and TZM-bl cells, respectively, as described in *Material and Methods*. The titers for 5-fold diluted VSV-Gpp and undiluted HIVpp were $3.4x10^6$ and $2.3x10^4$ $TCID_{50}$/mL, respectively. The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

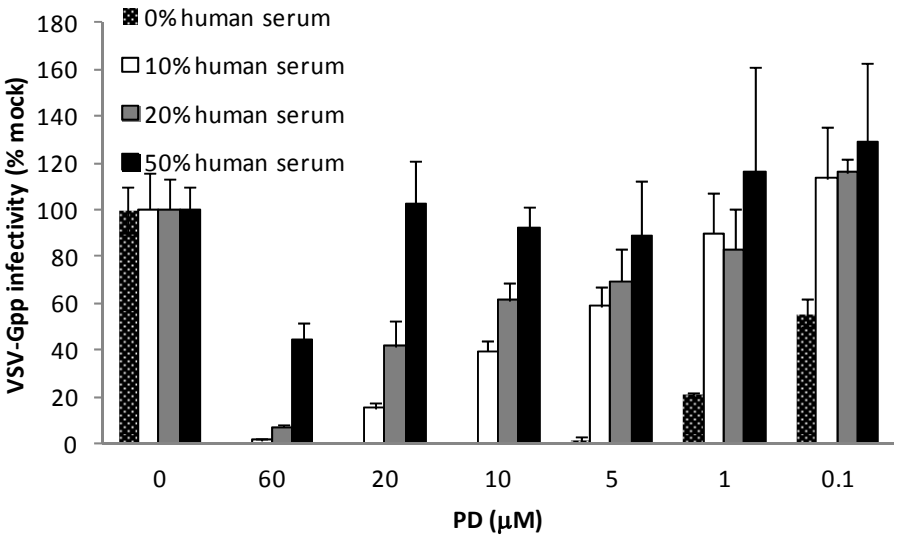

Fig. S6. The antiviral potency of PD is inhibited by the presence of human serum. VSV-Gpp was diluted 500-fold in medium containing different concentrations of human serum, incubated with appropriately diluted PD at 37 °C for 30 mins, and used to spinoculate Huh-7.5 cells at 4 °C as described in *Materials and Methods*. Cells were washed 4 times with fresh medium and incubated at 37°C/5% CO_2. Viral infectivity was determined by measuring the supernatant activity of the Gluc reporter 48 h later. The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

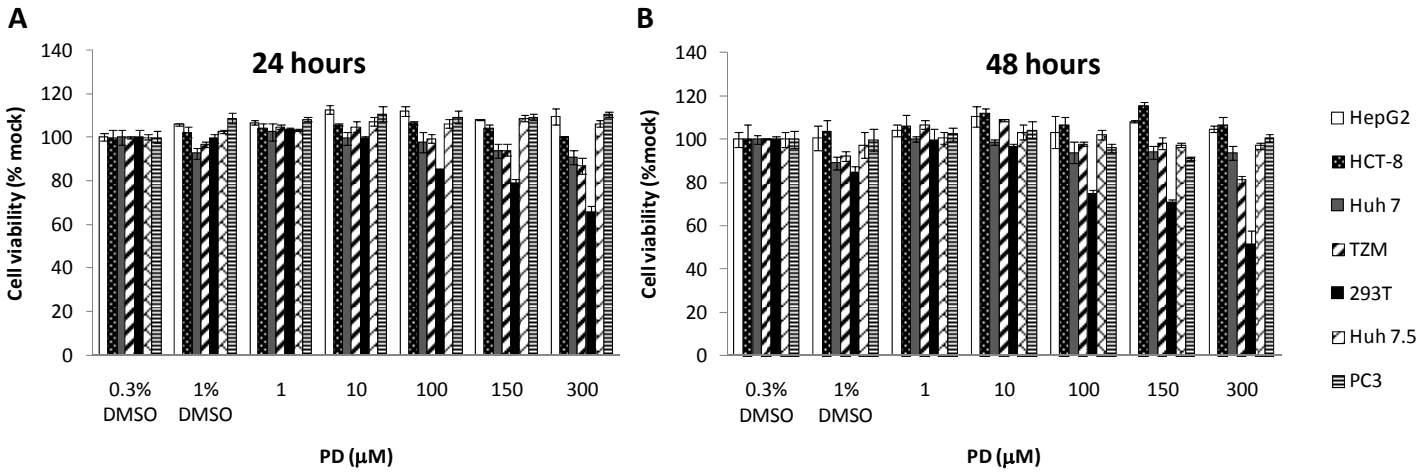

Fig. S7. Cytoxicity of PD 404,182 on different human cell lines. The cytotoxicity of PD was determined in the human cell lines HepG2 (hepatoma), HCT-8 (colon cancer), Huh-7 (hepatoma), Huh-7.5 (hepatoma), TZM (cervical cancer), PC3 (prostate cancer), and 293T (embryonic kidney). Cells were seeded in 96-well flat bottom tissue culture plates at 1.8×10^4 – 3.2×10^4 cells per well, where cell lines that divide faster were seeded at lower densities. After plating, cell culture supernatants were replaced with PD-containing medium, and cells were incubated at 37°C/5% CO_2. At 12 h intervals post initial treatment with compound, cell culture supernatants were removed and replaced with freshly prepared PD diluted in complete growth medium. At 24 h (**A**) and 48 h (**B**) post initial treatment with PD, cell viability was determined using CellTiter-Glo reagent. Cell culture supernatants were removed and replaced with 50 µL CellTiter-Glo reagent diluted 1:10 in ddH_2O. Microplates were then gently vortexed for 2 min and incubated at room temperature for an additional 8 min. Ten microliters of sample from each well was transferred to a white 96-well plate (Corning), and luminescence was measured in a Berthold Tristar LB 941 luminometer for 0.1 s. The error bars represent the mean ± SD of 2 independent experiments done in duplicate.

References

1. Chockalingam K, Simeon RL, Rice CM, Chen Z (2010) A cell protection screen reveals potent inhibitors of multiple stages of the hepatitis C virus life cycle. *Proc Natl Acad Sci U S A* 107:3764-3769.
2. Rice CM, Levis R, Strauss JH, Huang HV (1987) Production of infectious RNA transcripts from Sindbis virus cDNA clones: mapping of lethal mutations, rescue of a temperature-sensitive marker, and in vitro mutagenesis to generate defined mutants. *J Virol* 61:3809-3819.
3. Bick MJ*, et al.* (2003) Expression of the zinc-finger antiviral protein inhibits alphavirus replication. *J Virol* 77:11555-11562.
4. Brugger B*, et al.* (2006) The HIV lipidome: a raft with an unusual composition. *Proc Natl Acad Sci U S A* 103:2641-2646.