Supplemental Data

Figure S1

To verify transferrin receptor (CD71) expression on hCMEC/D3 cell line, the cells were harvested with M-PER Mammalian Protein Extraction Reagent (Pierce, Rockford, IL) supplemented with 0.2% v/v protease inhibitor cocktail P8340 (Sigma Aldrich, St. Louis, MO). Aliquots of cell lysate were added on 8 % SDS-polyacrylamide gel and electrotransferred onto a polyvinylidene difluoride membrane (Immobilon-P 0.45 µm, Millipore, Billerica, MA). Membranes were blocked with Tris-buffered saline containing 0.1% Tween 20 and 5% dry skim milk powder. Transferrin receptor and β-actin were detected with mouse CD71 monoclonal antibody (1:1000) and β-actin antibody (1:5000 dilution) respectively, followed by anti-mouse IgG (1:10000) as secondary antibodies. Proteins were visualized using enhanced chemiluminescence following the manufacturer instructions (Pierce, Rockford, IL). Prostate cancer cells PC3-M which were shown to express ten-fold of CD71 were used as a positive control.

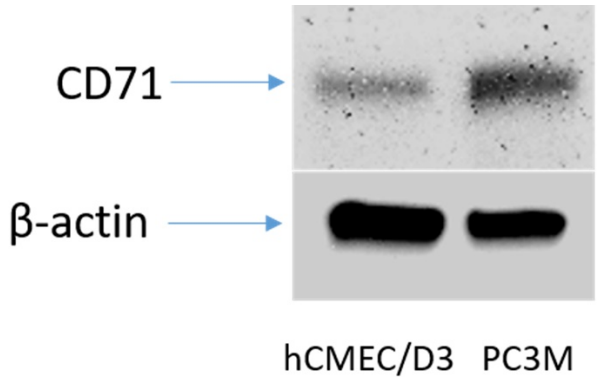

Figure S2

Male mice were randomized into three groups (N=3 per group per time point), and each was treated with either darunavir loaded Tf-nanoARV, free darunavir or normal saline (as vehicle control) via tail-vein injection. The first two formulations were given at 5 mg drug/kg dose level. At 4 or 24 hours after treatment, animals were sacrificed, dissected and various organs (including brain, liver, spleen, kidney, heart, lung) extracted for LC-MS measurement of darunavir. For each gram of tissue, 2 mL of extracting solution (3:1 of PBS: acetonitrile by volume) was added for homogenization. The mixture was ultracentrifuged under 150,000 g for 30 min. LC-MS detection was performed using API4000 LC-MS/MS system (SCIEX, Framingham, MA). The data of vehicle-treated animal brains served as the baseline control. Means±SD presented. **P*<0.05.