Supplemental Materials and Methods

Media preparation

All FBS samples were heat inactivated in 50 ml aliquots in polypropylene conical tubes (BD Falcon, Franklin Lakes, NJ) in a 56C water bath for 45 minutes. Samples were allowed to cool to room temperature and then frozen at -20C. 50 ml of heat inactivated serum was thawed overnight at 4C and then added to 500 ml Dulbecco's Modified Eagle Medium high glucose (D-MEM) (Thermo Scientific, Logan, Utah) along with 5.5 ml penicillin/streptomycin (5,000 units/ml and 5,000 μg/ml respectively) (Lonza, Walkersville, MD). All media was stored at 4C and heated for 30 minutes at 37C before use.

Cell culture

Human embryonic kidney 293T cells (HEK293) were split into five 10 cm tissue culture treated dishes and cultured for three weeks in one of the five serum samples. This was performed to make sure any effects of serum that required prolonged culture would be observed in the cells used for viral production. Cells were passaged twice per week for a period of three weeks in their respective serum sample. HT1080 human fibrosarcoma cells were used to determine foamy and lentiviral titers. All cells were maintained in a 37C incubator with 5% CO_2. Transfections and transductions were performed in triplicate using the same respective serum sample for both production and titering of viral vectors.

Foamy viral vector preparation

Dishes were coated with poly-l-lysine (Trevigen Inc., Gaithersburg, MD) diluted at a ratio of one to four in Dulbecco's Phosphate Buffered Saline (D-PBS) (Thermo Scientific,

Logan, Utah). HEK293 cells were harvested and plated in triplicate at a density of 5 x 10^6 cells per 10 cm dish per serum sample. The next day (day 1), foamy virus transfection was performed as previously described (Kiem *et al.*, 2010). Briefly three plates of 5 x 10^6 HEK293 cells per serum sample were transfected with phosphoglycerate kinase (PGK) promoter driven enhanced green fluorescent protein (EGFP) expressing vector plasmid, along with helper plasmid constructs for Gag, Pol, and Env. 2 ml of Serum-free D-MEM was added to the Plasmid DNA per plate. 1 µg/µl polyethylenimine (Polysciences Inc., Warrington, PA) was added at a three to one ratio per microgram of DNA. Dishes were cultured overnight for 17 hours.

On day two, transfection containing media was removed and 6 ml D-PBS was gently added to the plates. D-PBS was aspirated from the plate and 12 ml of D-MEM with the corresponding FBS sample was carefully added to the plates. Dishes were then incubated for 55 hours at 37C and 5% CO_2. The following day (day 3), HT1080 cells were harvested and plated at a density of 2.5 x 10^5 cells per well on a six well plate.

On day four, six hours prior to the final harvest, media was aspirated from the HT1080 cells and 1 ml D-PBS was gently added. After aspirating off the D-PBS, 2.5 ml of D-MEM containing the respective serum sample for each transfection was added to each well, making sure not to disturb the cell monolayer. Media was harvested from each transfected HEK293 dish and then separately filtered using a 0.45 µm filter. 50 µl of filtered media from each sample was aliquoted separately in order to be used to determine vector titer on HT1080 cells. These aliquots were frozen briefly at -80C and then quickly thawed in a 37C water bath. 20 µl from each aliquot of filtered media was added to the corresponding well in the six well plate that contained the respective serum sample for a total of three transduced wells per serum sample. Three days later

(day 7), transduced cells were collected, spun down for 5 minutes at 470g, re-suspended in 1 ml D-PBS with 2% FBS, and then analyzed for EGFP expression by flow cytometry.

Lentiviral vector preparation

Dishes were coated with poly-l-lysine diluted at a ratio of one to four in D-PBS. HEK293 cells were harvested and plated in triplicate at a density of 5 x 10^6 cells per 10 cm dish per serum sample. The next day (day 1), lentiviral transduction was performed as previously described (Trobridge *et al.*, 2010). Briefly, three plates of 5 x 10^6 HEK293 cells per serum sample were transfected with a PGK promoter driven EGFP expressing vector plasmid, with helper plasmid constructs for Gag and Pol, and vesicular stomatitis virus G glycoprotein (VSV-G) Env. 700 µl Serum-free D-MEM was added to the Plasmid DNA and 1 µg/µl polyethylenimine was then added at a three to one ratio per microgram of DNA. Dishes were cultured overnight for 17 hours. On day two, transfection containing media was removed and 6 ml D-BPS was gently added to the plates. D-PBS was aspirated and 10 ml D-MEM containing the different FBS sample lots and also supplemented with 0.97% 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid, N-(2-Hydroxyethyl)piperazine-N′-(2-ethanesulfonic acid) (HEPES) (Sigma, St. Louis, MO) and 0.97% sodium butyrate (Alfa Aesar, Ward Hill, MA) was added to the dishes and incubated for 8 hours. Media was collected from the plates and stored at 4C. 10 ml D-MEM with the appropriate FBS sample lot and 0.98% HEPES was gently added to the transduced HEK293 cell plates and cultured overnight for 18 hours.

On day three, HT1080 cells were harvested and plated at a density of 2.5 x 10^5 cells per well of a six well plate. Media was collected from the HEK293 plates and stored at 4C. 10 ml D-MEM with the appropriate FBS sample lot and 0.98% HEPES was added to the HEK293 plates

and cultured for 8 hours before the final media collection. Previously collected media for each transfection sample was pooled with the final collection sample. Pooled media for each transfection was then sterile filtered using a 0.2 μm filter. A 50 μl aliquot of filtered media from each transfection sample dish was frozen overnight at -80C to be used to determine viral titer on HT1080 cells.

The next day (day 4), six hours prior to transduction, media was aspirated from the HT1080 cells and 1 ml D-PBS was gently added. After aspirating off the D-PBS, 2.5 ml of D-MEM containing the respective serum sample and supplemented with 2.5 μl of 4 mg/ml protamine sulfate (MP Biomedicals, Solon, OH), was gently added to three wells for each serum sample to avoid disturbing the monolayer. The 50 μl aliquots of filtered media were quickly thawed in a 37C water bath to be used to determine vector titer on HT1080 cells. 20 μl from each aliquot was added to the corresponding well of the six well plate that contained the respective serum sample for a total of three transduced wells per serum sample. Three days later (day 7), transduced cells were collected, spun down for 5 minutes at 470g, resuspended in 1 ml D-PBS with 2% FBS, and then analyzed for EGFP expression by flow cytometry.

KIEM, H.P., WU, R.A., SUN, G., VON LAER, D., ROSSI, J.J., and TROBRIDGE, G.D. (2010). Foamy combinatorial anti-HIV vectors with MGMTP140K potently inhibit HIV-1 and SHIV replication and mediate selection in vivo. Gene therapy **17**, 37-49.

TROBRIDGE, G.D., WU, R.A., HANSEN, M., IRONSIDE, C., WATTS, K.L., OLSEN, P., BEARD, B.C., and KIEM, H.P. (2010). Cocal-pseudotyped lentiviral vectors resist inactivation by human serum and efficiently transduce primate hematopoietic repopulating cells. Mol Ther **18**, 725-733.