Supplemental Table I. Summary of Product Quality Control Testing.

Assay	MCB	Vector
Sterility	√	√
Mycoplasma	√	√
Endotoxin		√
Cell Culture Identification and Characterization	√	
Cocultivation of Producer cells-RCR S+/L-	√	√
EOP or Vector Supernatant RCR S+/L-	√	√
SC-1/Eco-RCR (XC plaque assay)	√	
In-vitro Viral Contaminants	√	√
In-vivo Inapparent Virus	√	
MAP Test	√	
In-vitro Bovine Virus	√	
In-vitro Porcine Virus	√	
Human and Simian Virus PCR Panel (SV-40, HIV I/II, HBV, HHV6, HHV7, HHV8, CMV, EBV, HTLV I/II, HPV B19, HCV, Adeno E1a)	√	
Transmission Electron Microscopy	√	
Vector Identity by Southern Blot	√	

Supplementa Table II. Validation of Residual Cell Dectection Assay.

Assay	*Initial Cell Density*[a] *(Cell/mL, x10^6)*	*% Viability*	*Final Cell Number*[b]	*Colonies Detected*[c]
Directly Plated[d]	1.00 ± 0.2	98	3	Yes
			5	Yes
			10	Yes
Centrifuged[e]	1.00 ± 0.2	98	3	Yes
			5	Yes
			10	Yes

[a] PG13 packaging cell line expressing F5M TCR suspensions containing 1 x 10^6 cell/mL were prepared and serially diluted in medium yielding the final concentration of 10 cell/mL. Cell counting and viability assessments were performed (n = 8). Results are reported as mean ± s.d.

[b] Calculated final cell number.

[c] Cells were allowed to grow for 7-10 days before being inspected visually for colonies or fixed with methanol and stained with crystal violet before being scored.

[d] Direct plated cells were diluted in a total of 10 mL of medium and plated into 10 cm tissue culture-treated dishes.

[e] Centrifuged samples were diluted in 100 mL final volume, centrifuged at 1000*g* for 10 min at 4^{o}C in sterile 250 mL centrifuge tubes. The upper 90 mL was removed by aspiration, the cells resuspended by trituration and plated into 10 cm^2 tissue culture-treated dishes.

Supplemental Table III. Viable Packaging Cell Detection During Gammaretroviral Vector Production.

Harvest Day[a]	*Viable cells Per RB (x 10^6)*[b]	*Viable cells Per Harvest (x 10^6)*[c]
1	0.25 ± 0.1	12.5 ± 0.1
2	0.5 ± 0.2	25.0 ± 0.2
3	1.73 ± 0.2	86.5 ± 0.2
4	2.57 ± 0.2	129 ± 0.2

[a] Standard 850 cm^2 roller bottles were seeded with PG13 cells at 4 x 10^4 cells/cm^2 (N = 3). At confluence, medium is exchanged (60 mL per bottle) and harvested and refed every 24h. The supernatant was centrifuged, the upper 50 mL aspirated and the remaining cell pellet was triturated and counted.
[b] Average viable residual cells (± SEM) per roller bottle based on cell counts.
[c] Total number of residual cells (± SEM) calculated for a 50 roller bottle production.

Supplementat Table IV. Validation of Cell Removal by Freeze/Thaw.

Initial Cell Density[a] *(Cell/mL, x 10^6)*	*% Viability*	*Final Cell Number*[b]	*Colonies Detected*[c]	*LRV*[d]
1.00 ± 0.2	98	50	0/3	1
		500	0/3	2
		5000	0/3	3
		50,000	2/3	None

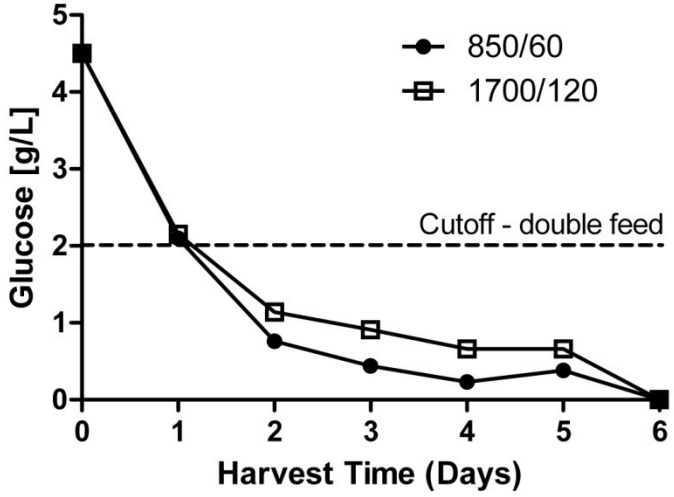
[a] PG13 packaging cell line expressing F5M TCR suspension containing 1 x 10^6 cell/mL was Prepared. Counting and viability assessments were performed (n = 8). Results are reported as mean ± s.d.

[b] Calculated final cell number. Cells were spiked into 100 mL of production medium and frozen at -80°C for 7 days. Samples were then thawed in a 37°C waterbath, centrifuged at 1000 x g. The top 90 mL was aspirated off and the remaining supernatant and pellet were triturated and plated into 10 cm^2 dishes.

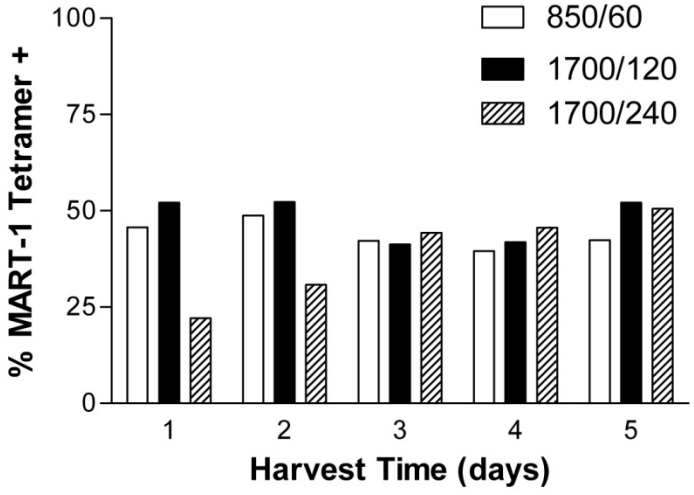
[c] Cells were allowed to grow for 7-10 days before being inspected visually for colonies or fixed with methanol and stained with crystal violet before being scored.

[d] Log-removal value; based on the detection assay sensitivity of 3-5 cells.

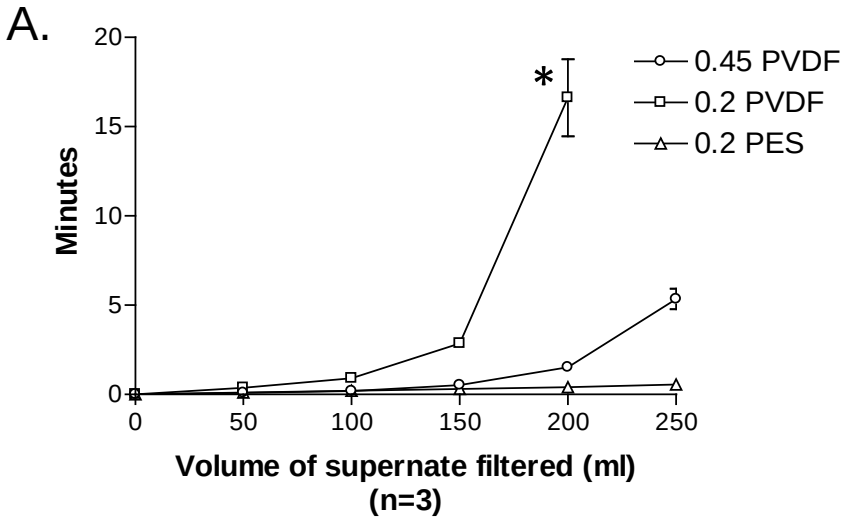
A.

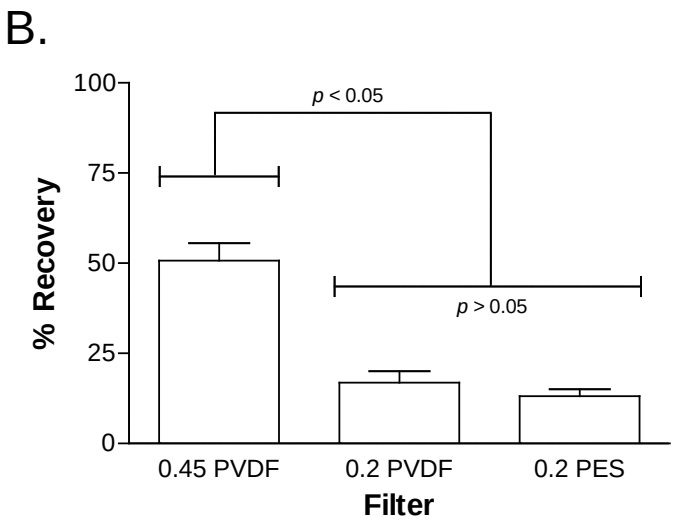


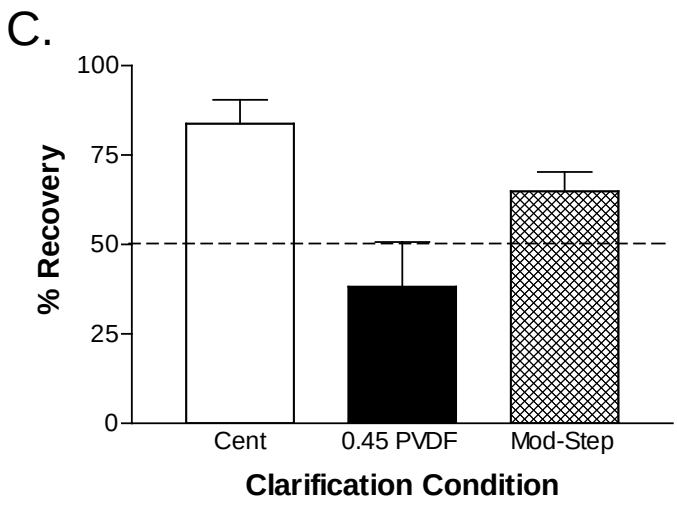
B.



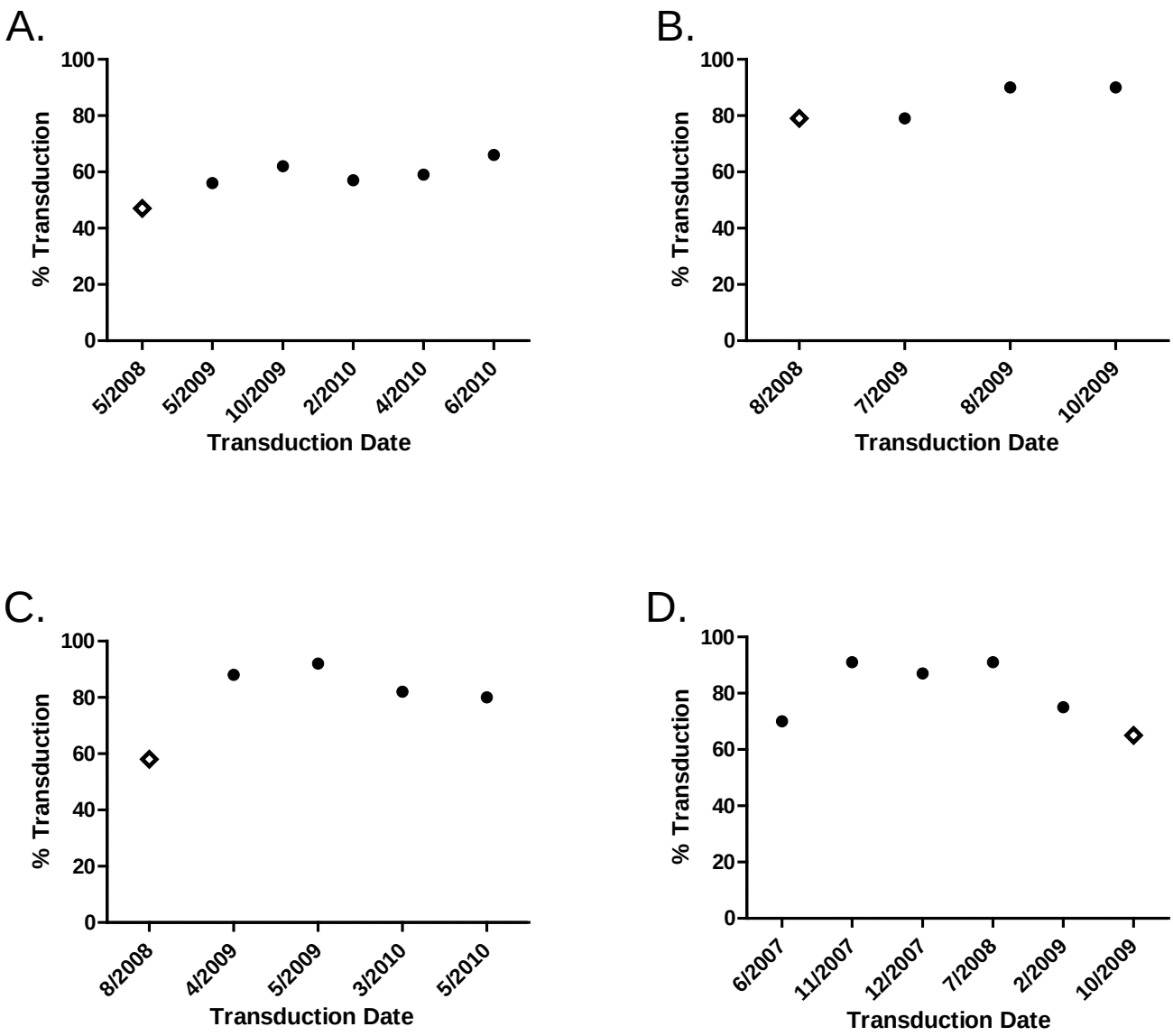
Supplemental Figure 1. Production of gammaretroviral vectors from PG13 packaging clones cultured in expanded surface roller bottles. (A) Glucose concentrations were recorded daily using the Accu-Chek Advantage glucose meter (Roche Diagnostics Corporation, Indianapolis, IN). (B) For determination of percentage of tetramer positive cells, transduced PBL were subjected to fluorescence activated cell sorting (FACS) using FITC-conjugated anti-CD3 antibody (BD Biosciences, San Jose, CA) and PE-conjugated peptide (MART-1_{27-35})/HLA-A*02 tetramers (Beckman-Coulter, Fullerton, CA). A representative experiment is shown.







Supplemental Figure 2. Comparison of methods for the clarification of gammaretroviral vector supernatants. Two hundred fifty mL of vector supernatant produced from PG13-F5mA2aB (1G12) was applied to 0.45m and 0.2m PVDF or 0.2m PES Stericup bottle top filters, respectively (Millipore, Billerica, MA). (A) Vector supernatant was vacuum filtered and time (min) was recorded at 50 mL intervals until filtration was complete or the filter clogged (*). Data are presented as $\pm$ the standard error of the mean for 3 separate experiments. (B) Vector recovery following filtration was calculated from the percentage of tetramer positive cells following PBL transduction as described. Data are presented as $\pm$ the standard error of the mean for 3 separate experiments each normalized to the pre-filtration value [0.45m PVDF ($p \leq 0.05$) vs. 0.2m PVDF or PES; One-way ANOVA]. No other significant differences were observed. (C) Vector recovery was compared for centrifugation, modified step-filtration and membrane filtration. For centrifugation, 250 mL of vector supernatant was clarified by centrifugation for 10 min at 1000 x g. The upper 200 mL was collected for analysis. For membrane filtration and modified step-filtration, 250 mL of vector supernatant was applied to either a 0.45m PVDF Stericup filter or a 40/150m dual screen filter followed by a Sepacell 500II filter (Baxter, Deerfield, IL) in series. The pre- and post-clarified products were collected and used for PBL transductions. Vector titers were normalized to the pre-clarification titer to calculate the % recovery. Data are from at least two separate experiments with error bars representing $\pm$ the standard error of the mean (One-way ANOVA, $p \geq 0.05$).



Supplemental Figure 3. Stability of clinical gammaretroviral vector recovery following cGMP manufacturing using modified step-filtration. At the specified time point, each vector was used to transduce a single patient PBL. For clinical transductions, PBL were transduced twice on days 2 and 3 post-OKT3 stimulation (filled circles). For titer evaluation post-production (see Figures 2 and 3), PBL were transduced only once on day 2 (open triangle). The percent transduction of the $CD3^+$ T cell population is shown. (A) CD19 CAR detection was determined using a biotin labeled polyclonal goat anti-mouse $F(ab)_2$ (Jackson Immunoresearch, West Grove, PA) followed by staining with streptavidin-PE (BD Pharmingen, San Diego, CA). CD19 CAR transduction efficiency was determined as the percentage of $CD3^+$/CD19 CAR positive cells; (B) CEA TCR transduction efficiency was measured as the percentage of $CD3^+$/murine b chain constant region (eBiosciences, San Diego, CA) positive cells; (C) 2G1 TCR expression was measured as the percentage of $CD3^+$/anti-Vb2 antibody positive cells (Beckman Coulter, Fullerton, CA). (D) A small subset of the total number of patients treated with TCR are shown. DMF5 transduction efficiency was measured as the percentage of $CD3^+$/MART-1 tetramer positive cells.