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Supplemental Information

High-Efficiency Lentiviral Transduction of Human CD34^{+} Cells in High-Density Culture with Poloxamer and Prostaglandin E2

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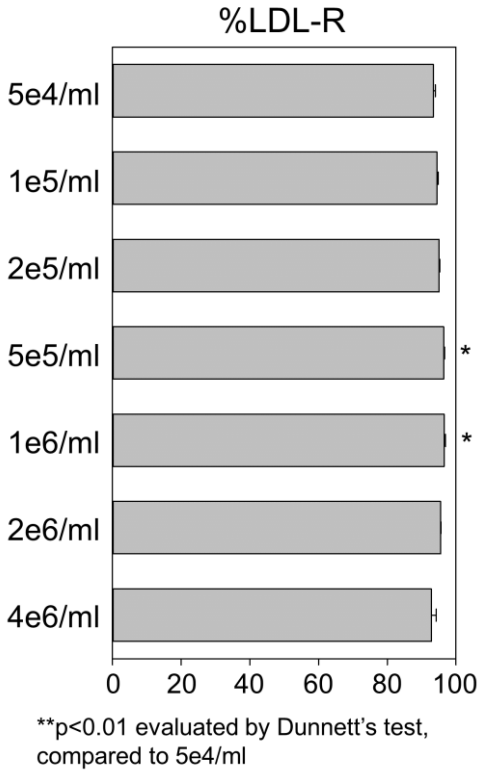
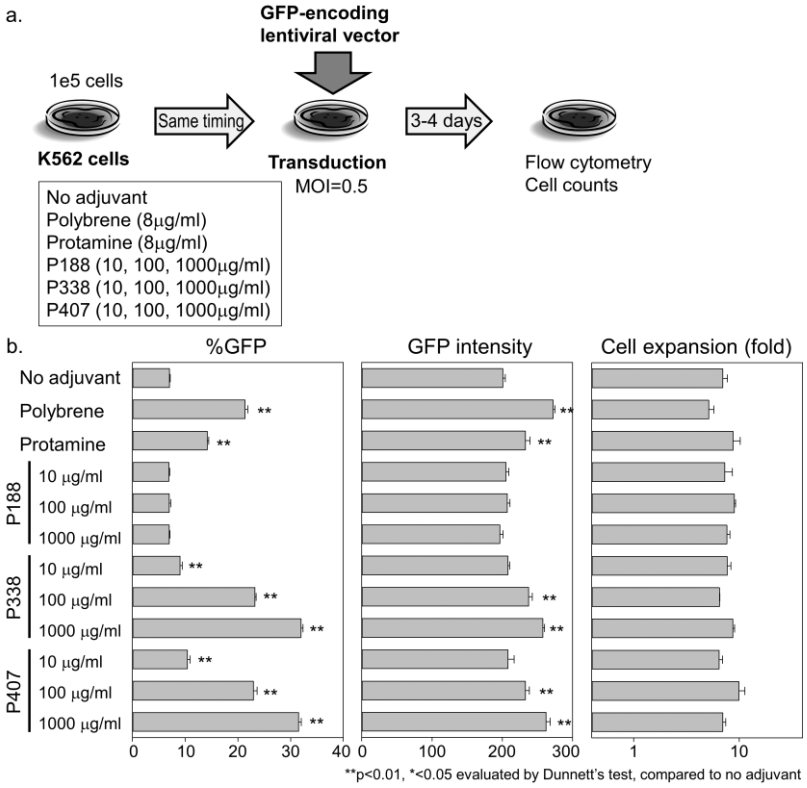
Supplementary figures

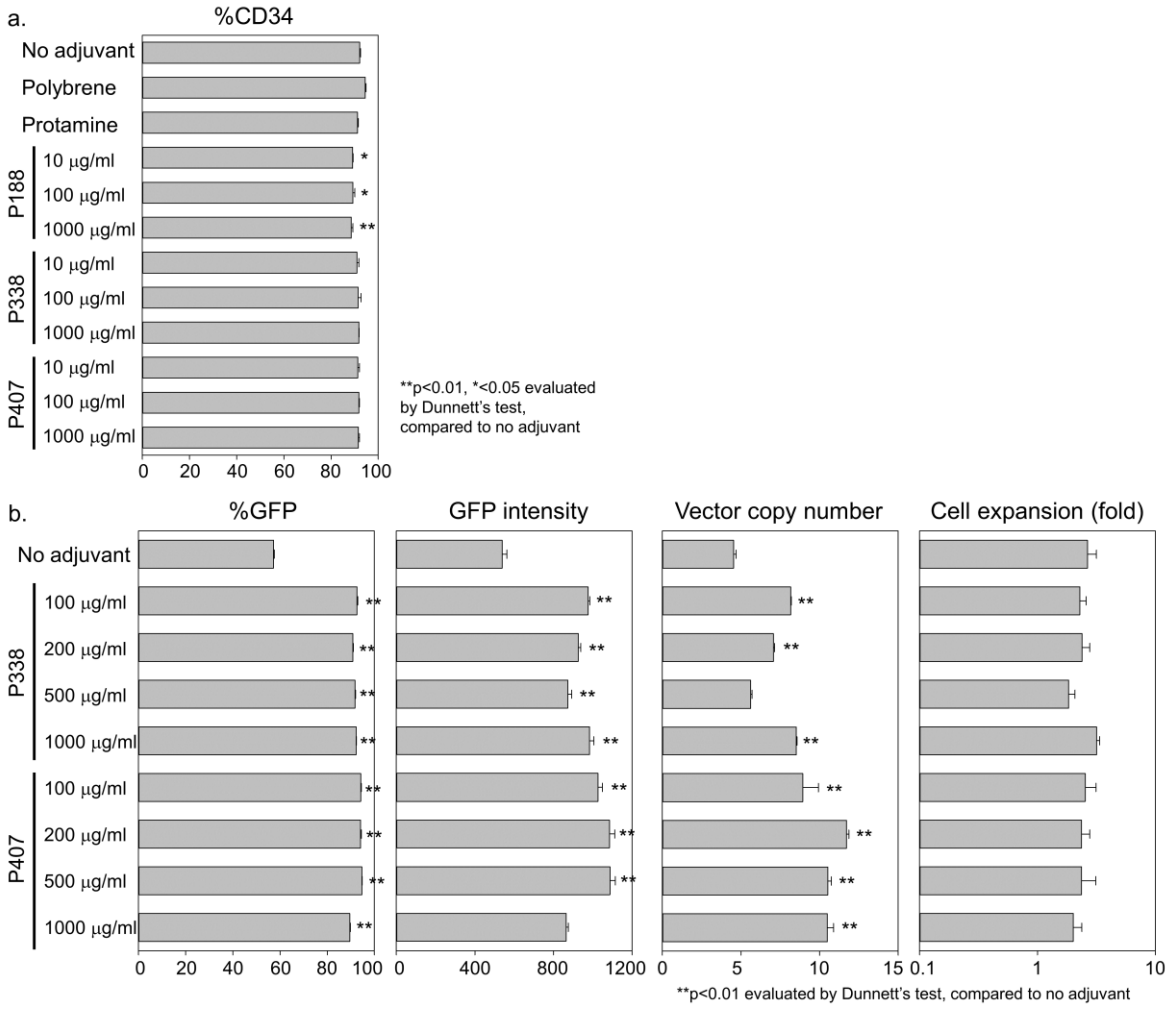
Supplementary figure 1. Similar low-density lipoprotein receptor (LDL-R) expression was detected in high-density CD34$^+$ cells. We evaluated LDL-R expression by flow cytometry at various cell densities of human CD34$^+$ cells after 1-day culture. Values: mean ± standard error. All experiments were performed in triplicate.



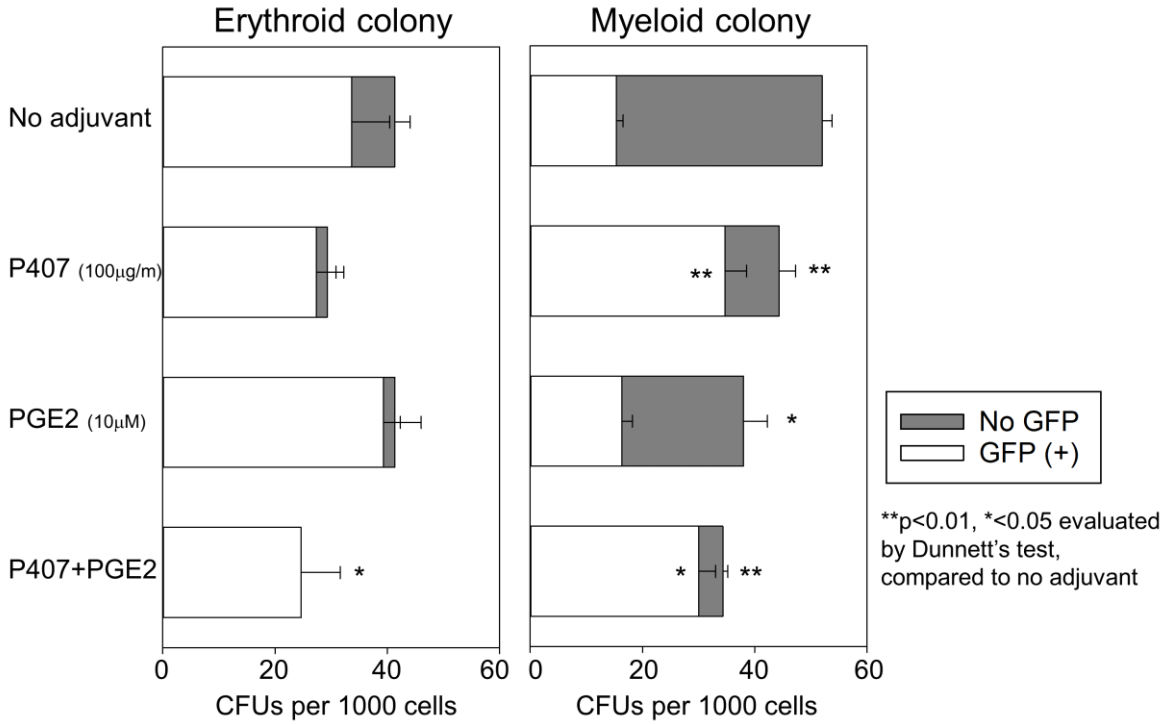
Supplementary figure 2. Addition of poloxamer to a K562 erythroleukemia cell line results in more efficient transduction with a lentiviral vector. (a) We transduced K562 cells (1e5/ml) with a GFP-expressing lentiviral vector at MOI 0.5 supplemented with various single reagents (potential adjuvants), including polybrene (8μg/ml), protamine (8μg/ml), P188 (10, 100, and 1000μg/ml), P338 (10, 100, and 1000μg/ml), and P407 (10, 100, and 1000μg/ml) for 1 day. %GFP (day 3 after transduction), GFP intensity (day 3), and cell counts (day 4) were evaluated 3-4 days after transduction. Values: mean ± standard error. All experiments were performed in triplicate.



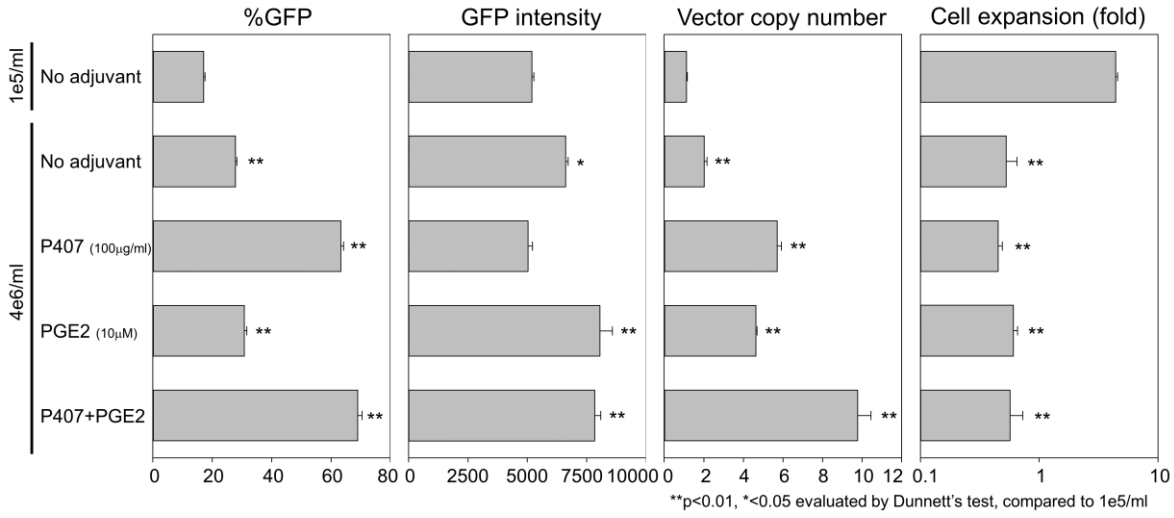
Supplementary figure 3. Addition of poloxamer to high-density CD34$^+$ cell culture results in more efficient transduction with a lentiviral vector. (a) After 1-day pre-stimulation, we transduced high-density CD34$^+$ cells (4e6/ml) at MOI 50 supplemented with various single reagents, using the same conditions described in Figures 3a and b. CD34 expression (%CD34) was evaluated 4 days after transduction. (b) After 1-day pre-stimulation, high-density CD34$^+$ cells (4e6/ml) were transduced with a GFP-expressing lentiviral vector at MOI 50 supplemented with P338 (100, 200, 500, and 1000μg/ml) or P407 (100, 200, 500, and 1000μg/ml) for 1 day. %GFP, GFP intensity, and cell counts were evaluated 4 days after transduction, and VCNs were evaluated 7 days after transduction. Values: mean ± standard error. All experiments were performed in triplicate.


a.
%CD34
No adjuvant
Polybrene
Protamine
P188
10 μg/ml
100 μg/ml
1000 μg/ml
P338
10 μg/ml
100 μg/ml
1000 μg/ml
P407
10 μg/ml
100 μg/ml
1000 μg/ml
0 20 40 60 80 100
**p<0.01, *<0.05 evaluated by Dunnett's test, compared to no adjuvant
b.
%GFP
GFP intensity
Vector copy number
Cell expansion (fold)
No adjuvant
P338
100 μg/ml
200 μg/ml
500 μg/ml
1000 μg/ml
P407
100 μg/ml
200 μg/ml
500 μg/ml
1000 μg/ml
0 20 40 60 80 100
0 400 800 1200
0 5 10 15
0.1 1 10
**p<0.01 evaluated by Dunnett's test, compared to no adjuvant

Supplementary figure 4. More efficient lentiviral transduction in CFUs results from high-density CD34⁺ cell culture with P407 and PGE2 supplementation. After 1-day pre-stimulation, we transduced high-density CD34⁺ cells with a GFP-expressing lentiviral vector at MOI 50 with P407 (100μg/ml), PGE2 (100μM), and a combination of P407 and PGE2. One day later, transduced cells were cultured in semi-solid media for 9 days, and CFUs and GFP positivity were evaluated by UV microscopy. Values: mean ± standard error. All experiments were performed in triplicate.



Supplementary figure 5. High-density culture with P407 and PGE2 improves lentiviral transduction in human CD34⁺ cells. (a) After 1-day pre-stimulation, human CD34⁺ cells were transduced with a GFP-expressing lentiviral vector at MOI 50 in our standard cell density culture (1e5/ml) without adjuvant, as well as high-density culture (4e6/ml) with P407 (100μg/ml) and/or PGE2 (100μM). All %GFP, GFP intensity, VCNs, and cell counts were evaluated at the same time point (6 days after transduction). Values: mean ± standard error. All experiments were performed in triplicate.



Supplementary figure 6. High-density culture with P407 and PGE2 improves lentiviral transduction in engrafting human CD34⁺ cells in xenograft mice. (a) After 1-day pre-stimulation, human CD34⁺ cells (2e5 cells/mouse) were transduced with a GFP-expressing lentiviral vector at MOI 50 in our standard cell density culture (1e5/ml) without adjuvant and high-density culture (4e6/ml) with/without P407 (100μg/ml) and PGE2 (100μM). One day later, transduced cells were transplanted into immunodeficient mice (NOD.Cg-Kit^{W-41J} Tyr^{+} Prkdc^{scid} Il2rg^{tm1Wjl}/ThomJ) 2 days after sublethal busulfan conditioning of 25mg/kg intraperitoneal injection (ip). (b) Twelve weeks after transplantation, we evaluated peripheral blood cells for human cell engraftment (human CD45-positive percentages), %GFP in human cells, and %GFP in whole cells (including both human and mouse cells). Values: mean ± standard error. 1e5/ml: n=3, 4e6/ml without adjuvant: n=1, and 4e6/ml with P407 and PGE2: n=2.