Supplementary Methods

Plasmid construction

The EcoRV-KpnI fragment of β-globin expression cassette in the TNS9.2 plasmid was inserted into the KpnI-HpaI fragment of the HIV1-based vector construct in the pCL20cMpGFP plasmid, to construct the pCL20c BGpGFP plasmid in which the globin gene was changed to GFP cDNA. The MluI-KpnI fragment of an MSCV-LTR promoter and GFP cDNA in the pCL20cMpGFP plasmid was, in reverse orientation, inserted into the EcoRI-KpnI fragment in the pCL20c BGpGFP plasmid, which included 3'UTR of β-globin gene as a polyadenylation (polyA) signal to construct the pCL20c rMpGFP plasmid. The 1.2kb XbaI-XbaI fragment of the full-length HS4 and 250b ScaI-HindIII fragment of core HS4

of the chicken β-globin gene in the pJC13-1 plasmid[38] was inserted into the deleted U3 region of 3'LTR in the pCL20c rMpGFP and the pCL20c BGpGFP plasmids. The core HS4 was inserted into the deleted U3 region of 3'LTR twice in the same orientation, to construct the tandem core HS4 (2x250b) insulated vectors. In the BGpGFP plasmids, we made mutations of internal polyA signals to increase viral titers for transduction of the human $CD34^+$ cells, as previously described.[39]

The HincII-SnabI fragment of a CMV enhancer from pCL20cMpGFP was inserted into the HpaI site of the upstream of an MSCV-LTR promoter in pCL20cMpGFP. The 1.2kb HS4, 2x250b HS4, and 250b HS4 were inserted into the ApaI site between the CMV enhancer and the MSCV-LTR promoter (Figure 4A).

Assay of p24 titers

The p24 titers of lentiviral vectors were evaluated by a p24 ELISA assay (Lenti-X™ p24 Rapid Titer Kit; Clontech, Mountain View, CA). The p24 concentrations of viral supernatants were assayed by the p24 ELISA assay, and the p24 titers were calculated by average ratios of p24 molecules to lentiviral particles (1ng p24 is equivalent to ~1.25×10^7 viral particles).