Supplementary Data

Supplementary Table S1. Removal of Plasmid DNA from pRRL-cPPT-MSCVU3-GFP-Wsin Vector Supernatant after Benzonase Treatment

Condition[a]	*Benzonase (U/ml)*[b]	*DNA in vector supernatant (ng/ml)*[c]	*Calculated residual DNA per dose (ng)*[d,e]
Room temperature, 1 hr	0	12.1 ± 1.4	1210.0
	25	UD	UD
	50	UD	UD
Room temperature, overnight	0	11.9 ± 1.7	1190.0
	25	UD	UD
	50	UD	UD
37°C, 1 hr	0	12.1 ± 1.7	1210.0
	25	UD	UD
	50	UD	UD

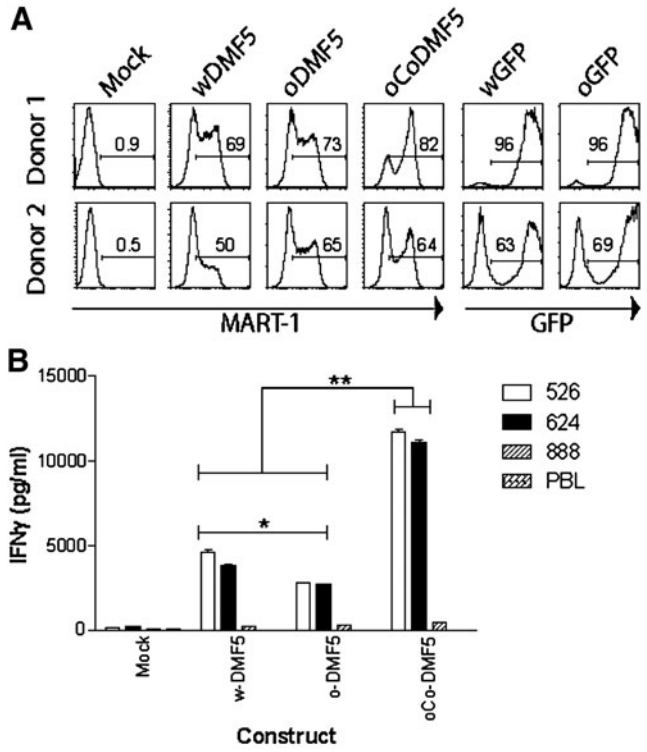
[a]Transiently generated pRRL-cPPT-MSCVU3-GFP-Wsin lentiviral vector was treated with 0, 25, or 50 units/ml Benzonase, under the conditions indicated.

[b]Units of Benzonase per milliliter (U/ml).

[c]Lentiviral vector supernatant was treated as indicated and residual plasmid DNA was quantitated by real-time PCR directed against the ampicillin resistance gene as described in Materials and Methods. Data presented are from at least three independent experiments.

[d]Calculated concentration of residual DNA per 100 ml of lentiviral vector product required for transduction of a single clinical product. UD, undetermined. The detection limit of the real-time PCR assay is 2.0 pg of DNA per milliliter.

[e]Limit for residual host cell DNA is 10 ng/dose (WHO guideline; Griffiths, 1997): Requirements for the use of animal cells as *in vitro* substrates for the production of biologicals, No. 50.



SUPPLEMENTARY FIG. S1. Improved receptor expression and function in PBL transduced with a LVV encoding the oPRE and a codon-optimized MART-1 TCR. (A) Comparison of DMF5 TCR expression in LVV constructs containing wPRE (wDMF5), oPRE (oDMF5) or oPRE and codon-optimized DMF5 (oCoDMF5). No differences in TCR expression were observed in PBL transduced with constructs containing the wPRE and oPRE. Codon-optimization of the DMF5 TCR slightly enhanced TCR expression compared to wDMF5 and oDMF5 (wGFP and oGFP were used as controls showing no difference in GFP expression). (B) Transduced PBL were evaluated for IFNγ release following culture with HLA-A*0201$^+$ MART-1$^+$ (526, 624) and HLA-A2$^-$ (888) tumor cell lines. PBL without any tumor cell line addition were also included as a negative control. PBL transduced with wDMF released significantly more IFNγ following culture with HLA-matched antigen-positive targets (*, $p < 0.5$). Use of oPRE in conjunction with a codon-optimized DMF5 TCR (oCoDMF5) resulted in a significant functional advantage over wPRE and oPRE as measured IFNγ release (**, $p < 0.05$). Data are from 3 separate patients PBL and error bars represent the standard error of the mean.