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Supplementary Materials for

Engineering human megakaryocytic microparticles for targeted delivery of nucleic acids to hematopoietic stem and progenitor cells

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Supplementary Materials

Supplementary figures

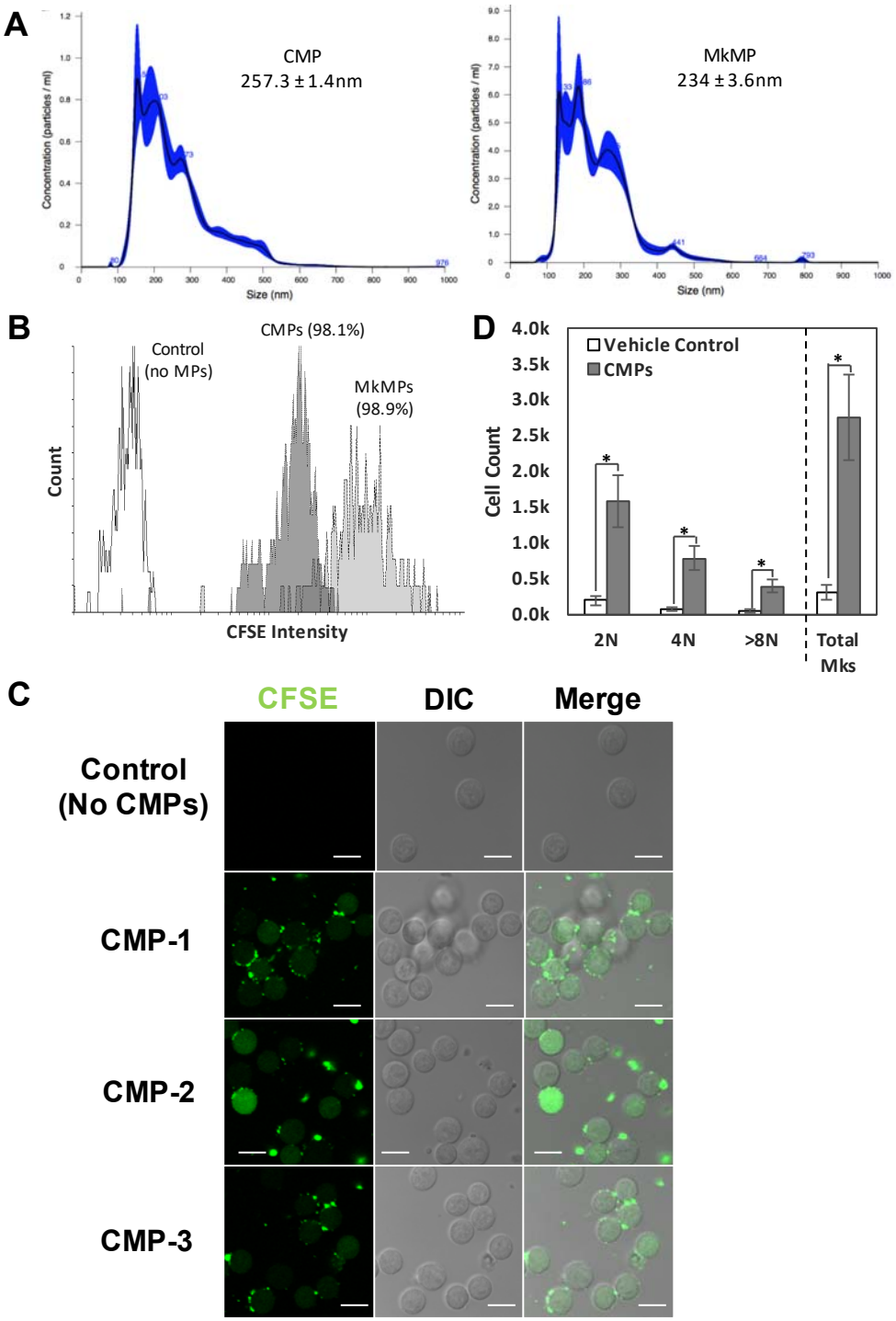

Fig. S1. Physical and functional characterization of CMP. (A) Size distribution measurement of CMPs and MkMPs by NTA. **(B** and **C)** CD34+ HSPCs were co-culture with CFSE-stained CMPs, MkMPs or vehicle control. After 30 minutes of co-culture, cells were harvested for **(B)** flow cytometry analysis on CFSE intensity, and **(C)** the uptake of CMPs with CFSE delivery to HSPCs were examined by confocal microscopy. **(D)** CMPs were co-cultured with CD34+ HSPCs and programed Mk differentiation. Cells were harvested at day 8 of co-culture and cell count of each ploidy class (2N, 4N, >8N), or total cell count were measured by flow cytometry. Scale bar in (C) represent 10 μM.

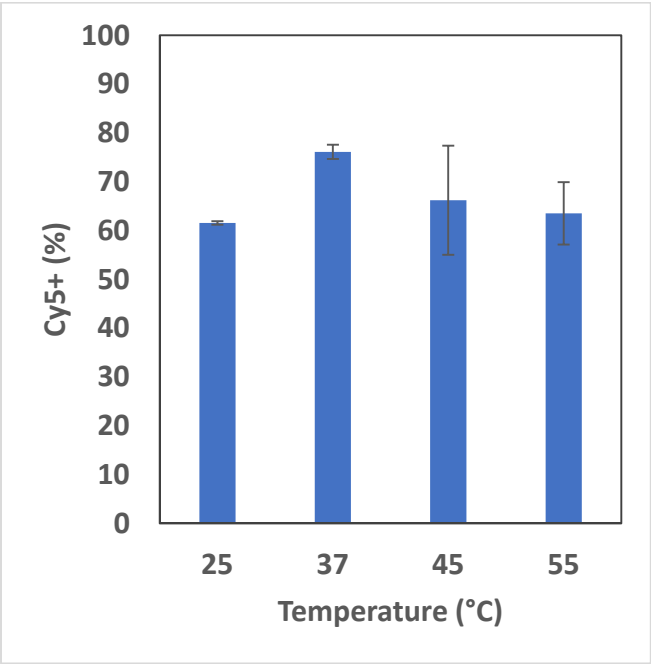

Fig. S2. The effect of electroporation temperature on pDNA loading efficiency. pGFPns was first stained with Cy5 fluorescent dye, then loaded into CMPs via electroporation at 100V, 100μF, in 2-mm cuvette at various temperature (25, 37, 45, or 55 °C). Cy5+ percentage of CMPs from each electroporation was quantified by flow cytometry analysis. Error bar represents SEM of three biological replicates.

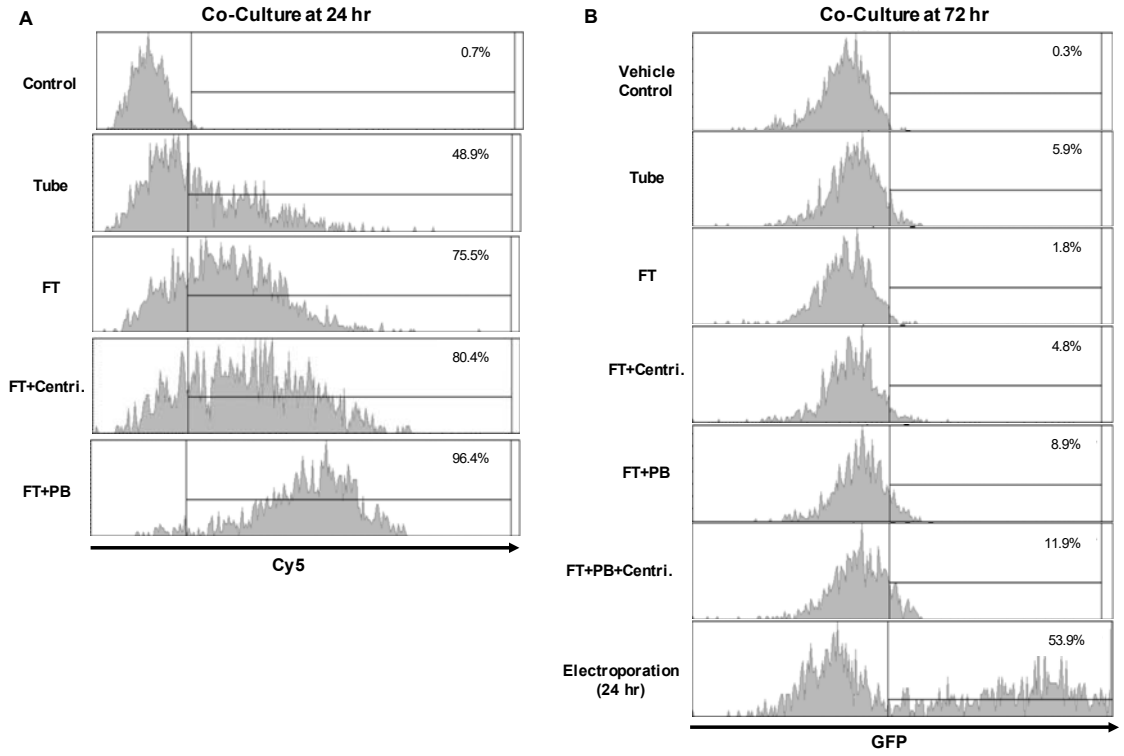

Fig. S3. Flow cytometric histograms. HSPCs were co-cultured with **(A)** Cy5-pGFPns-loaded CMPs, **(B)** pmaxGFP-loaded CMPs, and were harvested for flow cytometry analysis at **(A)** 24 hours for Cy5 signal, or **(B)** 72 hour for GFP expression. **(A)** Representative figures of Cy5 percentage associated with Fig. 2D. **(B)** Representative figures of GFP expression associated with Fig. 2F.

Supplementary tables

Table S1. Primers for amplification in PCR and qRT-PCR.

Gene	Forward Primer	Reverse Primer
GAPDH	CCCTTCATTGACCTCAACTACA	ATGACAAGCTTCCCGTTCTC
GFP	TCAAGATCCGCCACAACATC	GTGCTCAGGTAGTGGTTGTC
CMV	GGGTCATTAGTTCATAGCCCATA	GCCAAGTAGGAAAGTCCCATAA
c-myb	CTGCCTGGACGAACTGATAAT	TTGAAGACTCCTGCAGATAACC

Table S2. Estimation of DNA copies in loaded EVs from the literature.

EV Type	DNA Type	DNA Size	Loaded DNA copy per EV	Ref.
Exosome (85nm)	Linear	250 bp	462	(*16*)
MP (167nm)	Linear	250 bp	1347	
Exosome (85nm)	Plasmid	6 kb	1-2	
MP (167nm)	Plasmid	6 kb	8	
MP (257nm)	**Plasmid**	**6.29 kb**	**3455**	**Our Result**
MP (234 nm)	**Plasmid**	**3.49 kb**	**4264**	**Our Best Result**
MP (257 or 234 nm)	**Plasmid**	**9.8 kb**	**1700-2100**	**Estimation***

*Estimation of pDNA copy number in loaded-EVs from Latulippe *et al* (*35*) and our result.