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Supplemental Data

Generation of HLA-Universal iPSC-Derived Megakaryocytes and Platelets for Survival Under Refractoriness Conditions

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MATERIAL AND METHODS

Generation of HLA-Universal iPSCs Using a GMP-Conform Vector

To develop a system closer to good manufacturing practice (GMP) conditions, the expression cassette encoding shβ2m was cloned into the lentiviral vector pRRL.PPT.SF.DsRed.pre. The SF.DsRed expression cassette was replaced with the sequence encoding for the truncated form of the low-affinity nerve growth factor receptor (ΔLNGFR) [19] under the control of the elongation factor-1α-short (EFS) promotor. For the production of pRRL.PPT.EFS.dLNGFR.shβ2m.pre (shβ2m-pRRL) lentiviral particles, the safety-enhanced "third-generation" split plasmid packaging system [20] and the pMD2G plasmid encoding for VSVg envelope protein were used. Transduction efficiency was calculated by assessing the percentage of ΔLNGFR-expressing cells, quantified via flow cytometry using an anti-CD271 antibody labeled with APC (Miltenyi Biotec).

Generation and Characterization of HLA-Universal MKs and PLTs

For the differentiation of MKs and PLTs from shβ2m-pRRL-iPSCs the protocol as described in the main manuscript was applied. MK and PLT differentiation rates of iPSCs transduced either with shβ2m-pRRL or shβ2m-pLVTHm were compared.

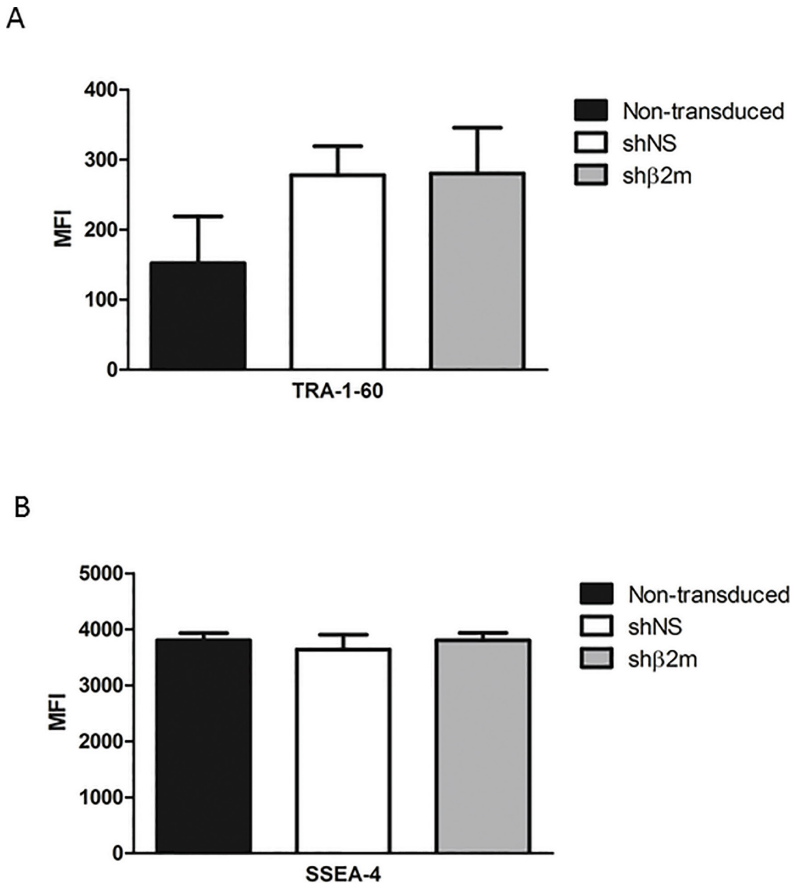
RESULTS

Generation of HLA-Universal MKs Using a GMP-Conform Lentiviral Vector

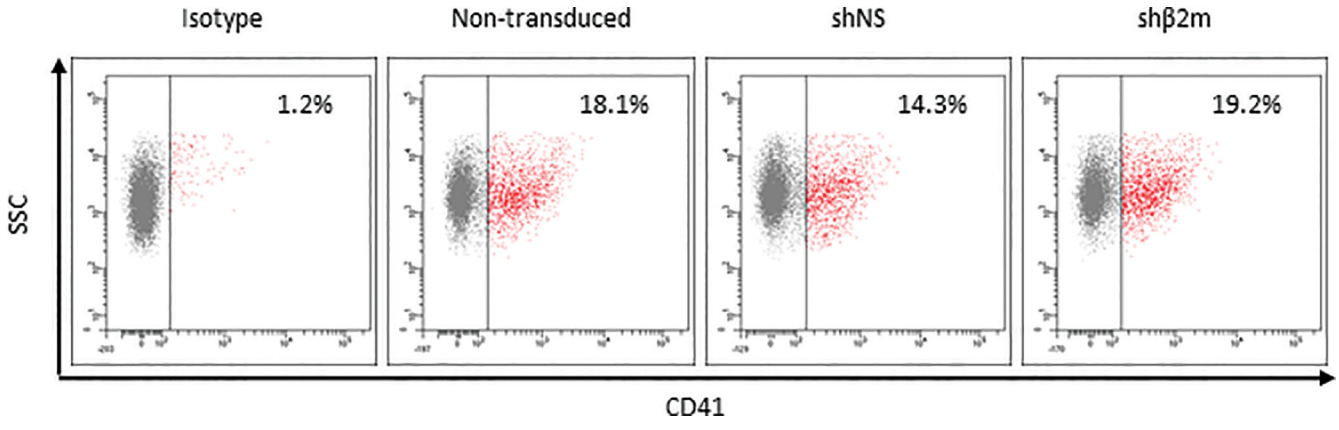
For the differentiation of iPSCs to MKs, cell lines expressing shβ2m either encoded in pLVTHm or pRRL are cultured for 33 d and were monitored every week for expression of MK markers. On d 19 47.5% ± 4.8 and 40.8% ± 17.7 $CD41^+$ $CD42a^+CD61^+$ cells were detected for shβ2m-pLVTHm and shβ2m-pRRL, respectively (Supplementary Figure 4A and 4B). For this experiment five independent differentiations comparing the two vectors were done separately, revealing no significant change in differentiation efficiency ($p = 0.493$).

Differentiation of HLA-Universal PLTs

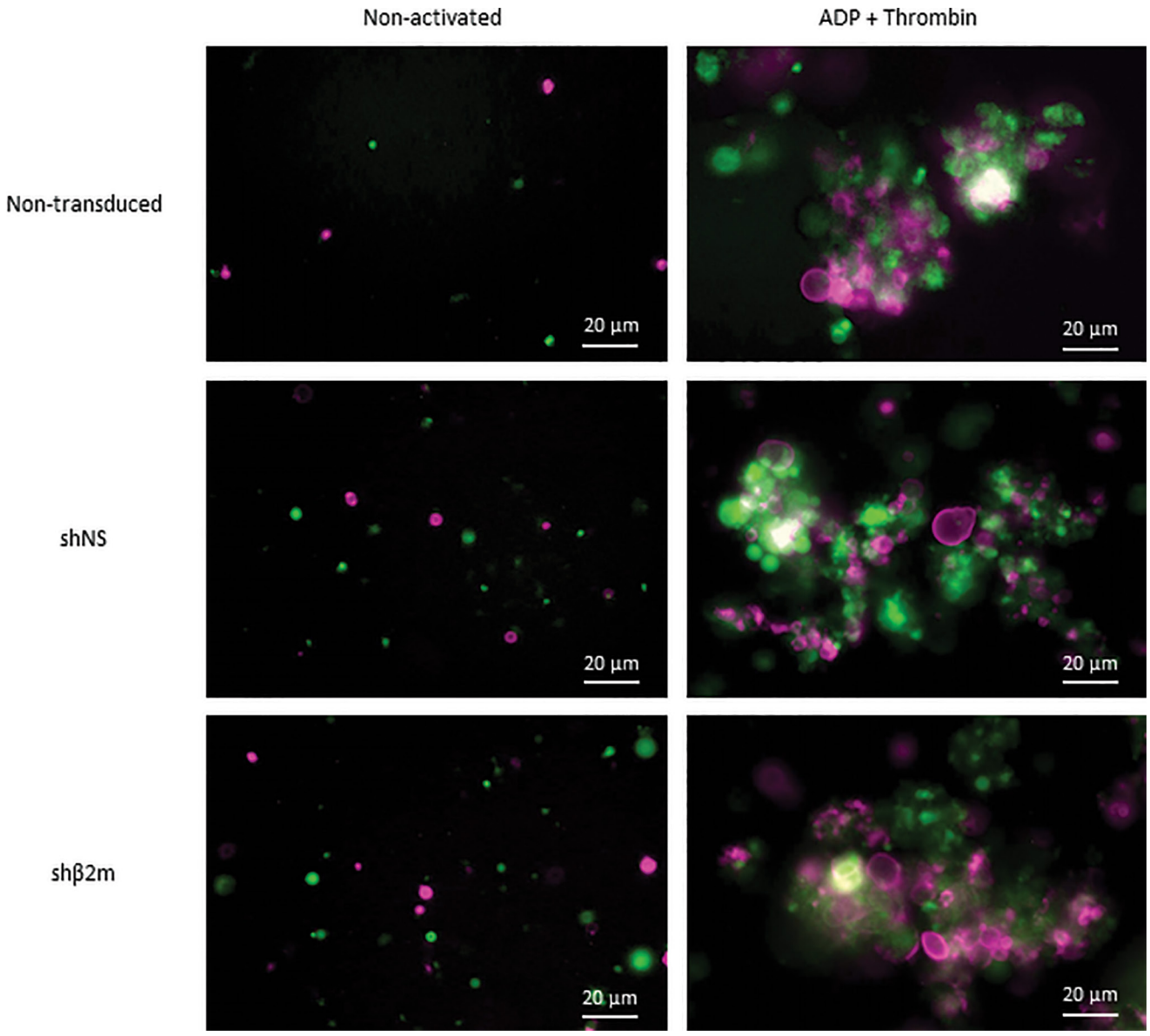
Starting on d 19 the supernatant of the ongoing differentiations was harvested and analyzed for the PLT content. On d 26, 76.2% ± 16.21 of the shβ2m-pLVTHm-derived and 80.6% ± 10.6 shβ2m-pRRL-derived $CD41^+$ cells were double positive for CD42a and CD61 (Supplementary Figure 5A and 5B). Comparing the differentiation efficiencies of the HLA-universal iPSCs with pLVTHm and pRRL, no significant change could be detected ($p = 0.602$).



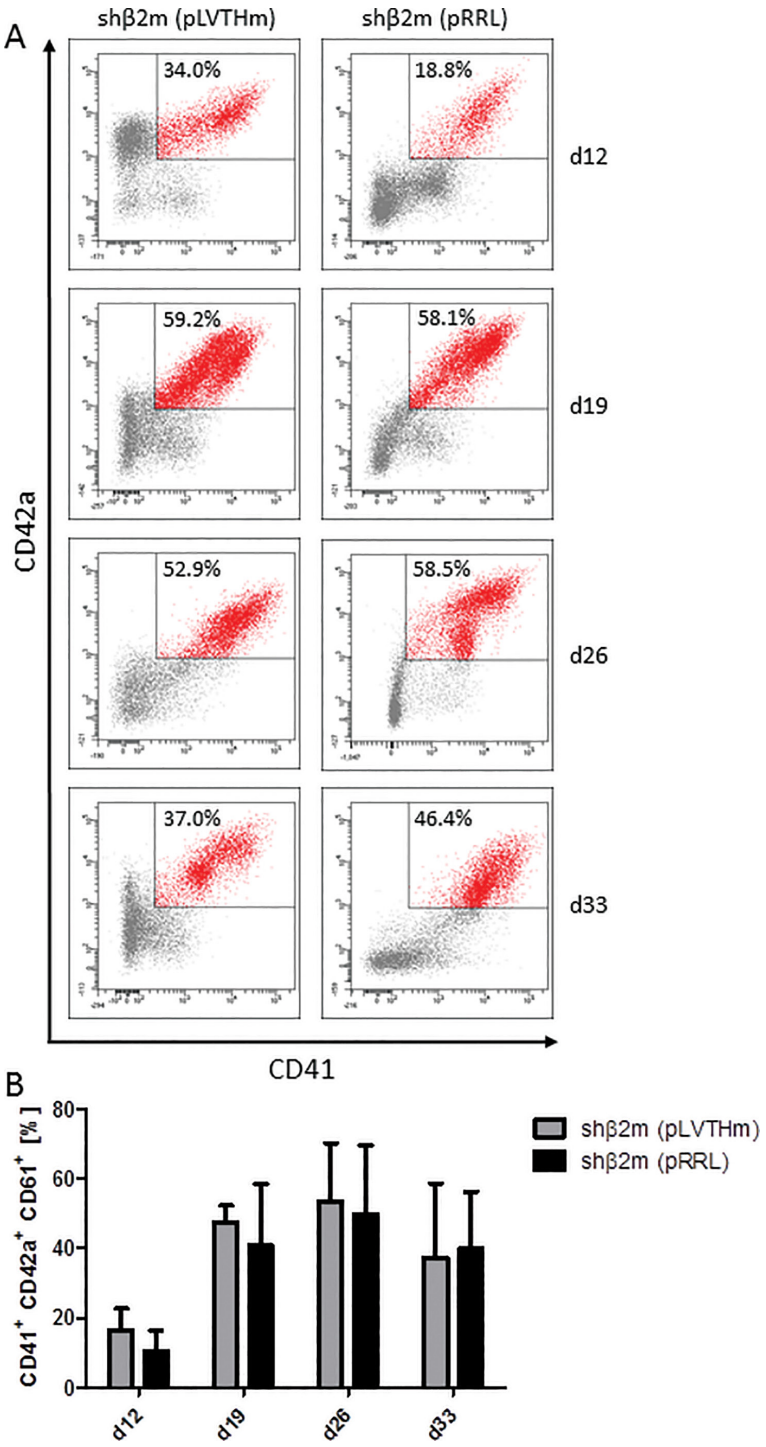
Supplementary Figure S1. HLA-universal iPSCs express pluripotency markers. iPSCs were transduced with shRNAs (NS or β2m-specific) encoded either by pLVTHm or pRRL.PPT.EFS. dLNGFR.pre (pRRL). The graph shows mean and SD of the mean fluorescence intensities of (A) TRA-1-60 and (B) SSEA-4 expression of HLA-universal iPSCs as detected by flow cytometry in three independent experiments.



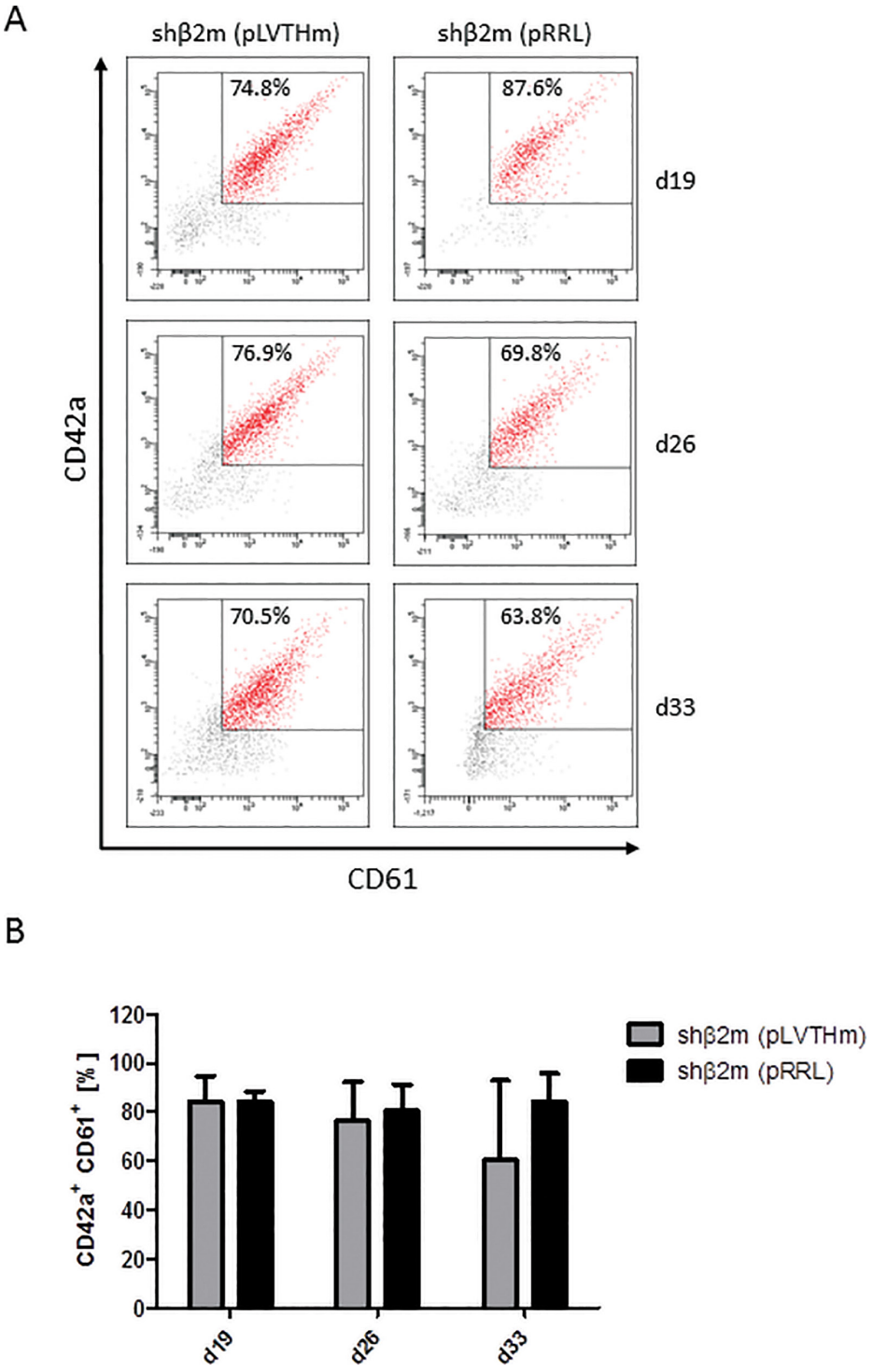
Supplementary Figure S2. Characterization of iPSC-derived platelets (PLTs). PLTs were derived from iPSCs, which were non-transduced or expressing either a shRNA targeting β2-microglobulin (shβ2m) or a non-specific shRNA (shNS). PLTs were analyzed weekly starting from d 19 for their expression of $CD41^+$ (GPIIb) population. This $CD41^+$ population was further analyzed for the co-expression of $CD42a^+$ (GPIX) $CD61^+$ (GPIIIa) (Figure 5). Representative flow cytometry dot plots are shown for each condition on d 19 of differentiation.



Supplementary Figure S3. HLA-universal platelets (PLTs) aggregate after stimulation. HLA universal PLTs derived from iPSCs were harvested and split in two fractions; one was labeled with FITC-conjugated anti-CD61 (GPIIIa) antibody (green) and the other with APC-conjugated anti-CD61 antibody (red). After staining, cell fractions were mixed together and again divided in a sample for stimulation with PLT agonists (ADP + thrombin) and a sample for control (non-stimulated). The figure shows fluorescence microscopy images before and after stimulation of PLTs derived from non-transduced iPSCs as well as from transduced iPSCs either expressing shNS or shβ2m.



Supplementary Figure S4. Characterization of megakaryocytes (MKs) derived from shβ2m-expressing iPSCs. The iPSCs transduced with either pLVTHm or pRRL were cultured for 33 d as described above and analyzed for MK differentiation on four time points (once a week). (A) The MK markers CD41 (GPIIb) and CD42a (GPIX) were used to determine the amount of MKs in the population. Representative flow cytometry dot plots are shown for shβ2m-expressing MKs (pLVTHm and pRRL) for each time point. (B) Frequencies of $CD41^+$ $CD42a^+$ $CD61^+$ cells detected by flow cytometry are shown with mean and SD of four independent experiments.



Supplementary Figure S5. Characterization of platelets (PLTs) derived from shβ2m-expressing iPSCs. The HLA-universal iPSCs (shβ2m-pLVTHm or shβ2m-pRRL) were cultured for 33 d and the supernatant was analyzed for PLTs at three time points starting at d 19. (A) PLTs were identified upon the expression of the typical PLT markers CD41 (GPIIb), CD42a (GPIX), and CD61 (GPIIIa). Therefore the $CD41^+$ population was selected and the amount of $CD42a^+CD61^+$ cells was determined. (B) Mean and SD of $CD42a^+CD61^+$ cells of four independent experiments.