

Down syndrome-related transient abnormal myelopoiesis is attributed to a specific erythro-megakaryocytic subpopulation with GATA1 mutation

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Supplemental Materials and methods

Establishment of TAM-iPS

To establish a PSC-based *in vitro* model representative of the hematological abnormalities observed in TAM, CD41⁺ blast cells were sorted using a FACS Aria II instrument (BD Biosciences, San Jose, CA, USA). Episomal plasmid vectors carrying reprogramming factors were introduced into blasts using the Amaxa Human CD34 cell Nucleofactor Kit (Lonza, Germany). After electroporation, cells were plated on BD Falcon tissue culture plates (#3516, CORNING, New York, USA) coated with 0.25 $\mu\text{g}/\text{cm}^2$ LM511-E8, which is a recombinant short fragment of laminin 511 (#892011, Nippi, Osaka, Japan), in StemSpan-ACF medium (STEMCELL Technologies, Vancouver, BC, Canada) containing 100 ng/mL SCF (#255-SC-010, R&D), 100 ng/mL TPO (#288-TP-005, R&D), 100 ng/mL Flt-3 Ligand (#308-FK-005, R&D), 50 ng/mL IL-6 (#206-IL-010, R&D), and 10 ng/mL IL-3 (#203-IL-010, R&D). The medium was changed to StemFit AK02N medium (Ajinomoto, Tokyo, Japan) gradually.

Maintenance of human PSCs

PSCs were maintained in StemFit AK02N medium on BD Falcon tissue culture plates coated with 0.25 $\mu\text{g}/\text{cm}^2$ LM511-E8. At each passage, the cells were treated with 0.5 x TrypLE select (#12563011, Thermo, Waltham, MA, USA) for 3 min at room temperature, harvested in AK02N medium with 10 μM Rho-Associated Protein Kinase Inhibitor Y-27632 (#08945-71, Nacalai Tesque, Kyoto, Japan), and re-plated on plates coated with LM511-E8 at a density of 265 cells/ cm^2 .

Transcription activator-like effector nucleases (TALEN) genome editing

To correct the mutation of *GATA1* gene, we used TALEN genome editing. We set the TALEN targeting site at intron 2 of *GATA1* gene and constructed TALEN plasmids using the Platinum Gate TALEN Kit (Addgene, Kit #1000000043) with a CAG promoter-driven destination vector. The right and left target sequences used are as follows: right, 5'-TCTGCACTTAACTAGGGA-3'; left, 5'-TGGAAGCTTCTCAAATGG-3'. For TALEN genome editing, PSCs were dissociated with TrypLE Select, and 1×10^6 cells were transfected with 2 μg of each TALEN

plasmid and 6 µg of the targeting vector plasmid in an N1 cuvette using the NEPA21 Super Electroporator (NEPAGENE) following the manufacturer's instruction. Then the PSCs were replated onto BD Falcon tissue culture plates coated with LM511-E8 with AK02N medium and 10 µM Y-27632. Cells were selected with 100 mg/mL G418 (Wako) 24 h after the transfection, and surviving clones were isolated 13 days after the drug selection. Selected clones were dissociated with TrypLE Select, and 1×10^6 cells were transfected with Cre expression plasmid using FuGene HD Transfection Reagent (Promega) following the manufacturer's instruction. Cells were selected with 500 ng/mL puromycin (InvivoGen) 2 days after the transfection. Four days after the transfection, the surviving cells were dissociated and expanded.

Monolayer hematopoietic differentiation system

First, the PSCs were plated onto LM511-E8-coated plates at a density of 2.7 to 3.6 cells/cm² and maintained as described in the previous subsection. When the undifferentiated colonies reached 750 to 1000 µm in diameter, the culture media was replaced with Essential-8 medium (#A1517001, Thermo) containing 80 ng/mL BMP4 (#314-BP-010, R&D), 80 ng/mL VEGF 165 (#293-VE-010, R&D), and 2 µM GSK-3 inhibitor CHIR99021 (#038-23101, Wako, Osaka, Japan). The media was changed every two days as follows. Day 2: Essential-6 medium (#A1516401, Thermo) containing 80 ng/mL VEGF, 25 ng/mL bFGF (#064-05381, Wako), 2 µM ALK5 inhibitor SB431542 (#031-24291, Wako), and 50 ng/mL SCF; Day 4: Stemline-II medium (#S0192, Sigma-Aldrich, St. Louis, MO, USA) containing 80 ng/mL VEGF, 50 ng/mL each of SCF, IL-6, IL-3, and Flt-3 Ligand, and 5 ng/mL TPO; and Day 6: Stemline-II containing 50 ng/mL each of SCF, IL-6, IL-3, and Flt-3 Ligand, 5 ng/mL TPO, and 10 U/mL EPO (#329871, Merk, Darmstadt, Germany). On day 9, the cells were harvested by flushing out colonies with PBS and filtering them through a 40 µm cell strainer. The collected cells were adjusted to 1×10^4 cells/cm² and transferred to lineage-specific suspension culture, which consisted of Stemline-II medium with 50 ng/mL each of SCF and IL-6, and either 10 U/mL EPO (for erythroid differentiation) or 20 ng/mL TPO (for megakaryocytic differentiation). On day 13, half the culture volume of fresh media containing 150 ng/mL SCF and either 30 U/mL EPO (for erythroid lineage) or 60 ng/mL TPO (for megakaryocytic lineage) was added to the culture.

Progenitor assay

For the progenitor assay, each HPC subpopulation sorted on day 9 was adjusted to 5×10^4 cells/mL and maintained under the appropriate lineage-specific suspension culture condition. After one week, the cells were counted and subjected to cell surface marker analysis.

RNA extraction and real-time quantitative RT-PCR

RNA samples were initially prepared using silica gel membrane-based spin-columns (RNeasy Kit; Qiagen, Valencia, CA, USA) following the manufacturer's instructions. Extracted RNAs were subjected to reverse transcription followed by cDNA amplification following a previously reported method with minimal modification^{1, 2}. Predesigned assays were used for the targeted detection of hematopoietic differentiation-related genes (PrimeTime predesigned qPCR assays; Integrated DNA Technologies, Coralville, IA, USA; see Supplemental Table 4), and quantitative RT-PCR experiments were performed with the StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA) using FastStart Universal Probe Master (#04913949001, Roche, Basel, Switzerland) following the manufacturer's instructions. Quantitative assessment of the gene expressions was performed using the standard Δ CT method. A combination of genes including GUSB, HPRT1, PGK1, ACTB, GAPDH, TBP, B2M, and PPIA served as multiple references.

Analysis of real-time PCR data

For principle component analysis (PCA), data sets were filtered by the criterion requiring positive values in at least one sample and subjected to variance-stabilizing transformation and interquartile range filtering, followed by upper-quartile normalization. Next, individual analyses were performed in R using the `prcomp` function after removing unwanted variations between clones by applying the open-source package "RUV-seq"³, and the results were visualized by the "Scatterplot3D" package obtained from the Bioconductor Project. Gene sets specifically relevant to the phenotypes of interest were extracted according to the correlations of the factor loadings between each gene and phenotype in a factorical space given in the PCAs, in which each phenotype was assigned as categorical data⁴. GENEMANIA⁵ and ReactomeFI⁶ followed by ClueGO⁷ plugin in Cytoscape (<http://www.cytoscape.org>) were used to visualize the network.

Ethics

Peripheral blood blast cells used for the iPSC generation were obtained from a Down syndrome patient (ID: CiRA12345 at Kyoto University and 778 at Hirosaki University) with written informed consent from the patient's guardians in accordance with the Declaration of Helsinki. The use of human ESCs was approved by the Ministry of Education Culture, Sports, Science and Technology of Japan (MEXT). This study was approved by the Ethics Committee and the recombinant DNA Experiments Safely Committee of Kyoto University. All methods were performed in accordance with the relevant guidelines and regulations.

May-Giemsa staining and microscopic observation

Phase contrast images of the culture were obtained by using BIOREVO BZ-9000 (Keyence) through a CFI Plan Fluor DL 4x objective lens (Nikon). For the microscopic observations of blood cells, floating cells were centrifuged onto PLATINUMPRO glass slides (Matsunami glass, Osaka, Japan) by using a Shandon Cytospin 4 Cytocentrifuge (Thermo, Pittsburgh, PA) and analyzed by BIOREVO BZ-9000 after May-Grunwald and Giemsa staining (Merck Millipore). A PlanApo 40x/0.95 objective (Nikon) and the BZ-II Viewer software program (Keyence) were used for the image acquisition.

Chromosome analysis

PSCs were cultured on 6-cm dishes until confluent and fixed with KaryoMAX (Thermo). Q-banding analyses were performed at Chromocenter (Kobe, Japan).

Sanger sequencing

Genomic DNA was extracted from the cells by using the PureLink Genomic DNA Kit (Invitrogen, Carlsbad, CA, USA) and subjected to PCR with the primers 5' -TTCTGAGAAAGTTAGAGGGCAAGATACAACAG-3' (forward) and 5' -GTACAGGGGTTCTATCTGGTATAAGTTTCTT-3' (reverse). After treatment with USB[®] ExoSAP-IT (Affymetrix, SC, CA), the amplicons were applied for Sanger sequencing, which was performed with the BigDye Terminator[®] v3.1 Cycle Sequencing Kit (Applied Biosystems, Carlsbad, CA). The products were

purified using the BigDye XTerminator[®] Purification Kit (ThermoFisher) and read by a 3500xL Sequencer (Applied Biosystems). Data were analyzed using Codon Code Aligner software (CodonCode Corporation) and 4Peaks (A. Griekspoor and Tom Groothuis).

Flow cytometry and antibodies

Flow cytometric analyses and cell sorting were performed using a FACS AriaII and FlowJo software (Flowjo LLC, Ashland, OR, USA). The following antibodies were used: BV421-conjugated anti-human CD235a (#562938) and BV605-conjugated anti-human CD34 (#343530), FITC-conjugated anti-human CD45 (#304006, BD Horizon[™], San Diego, CA, USA), and PE-conjugated anti-human CD42b (#303906), APC-conjugated anti-human CD71 (#334108), Alexa Flour[®] 647-conjugated anti-human CD309 (#338909), PE-Cy7-conjugated anti-human CD43 (#343208), APC-Cy7-conjugated anti-human CD41 (#303716), and PerCP-Cy5.5-conjugated anti-human CD11b (#301328), which were all purchased from BioLegend (San Diego, CA, USA).

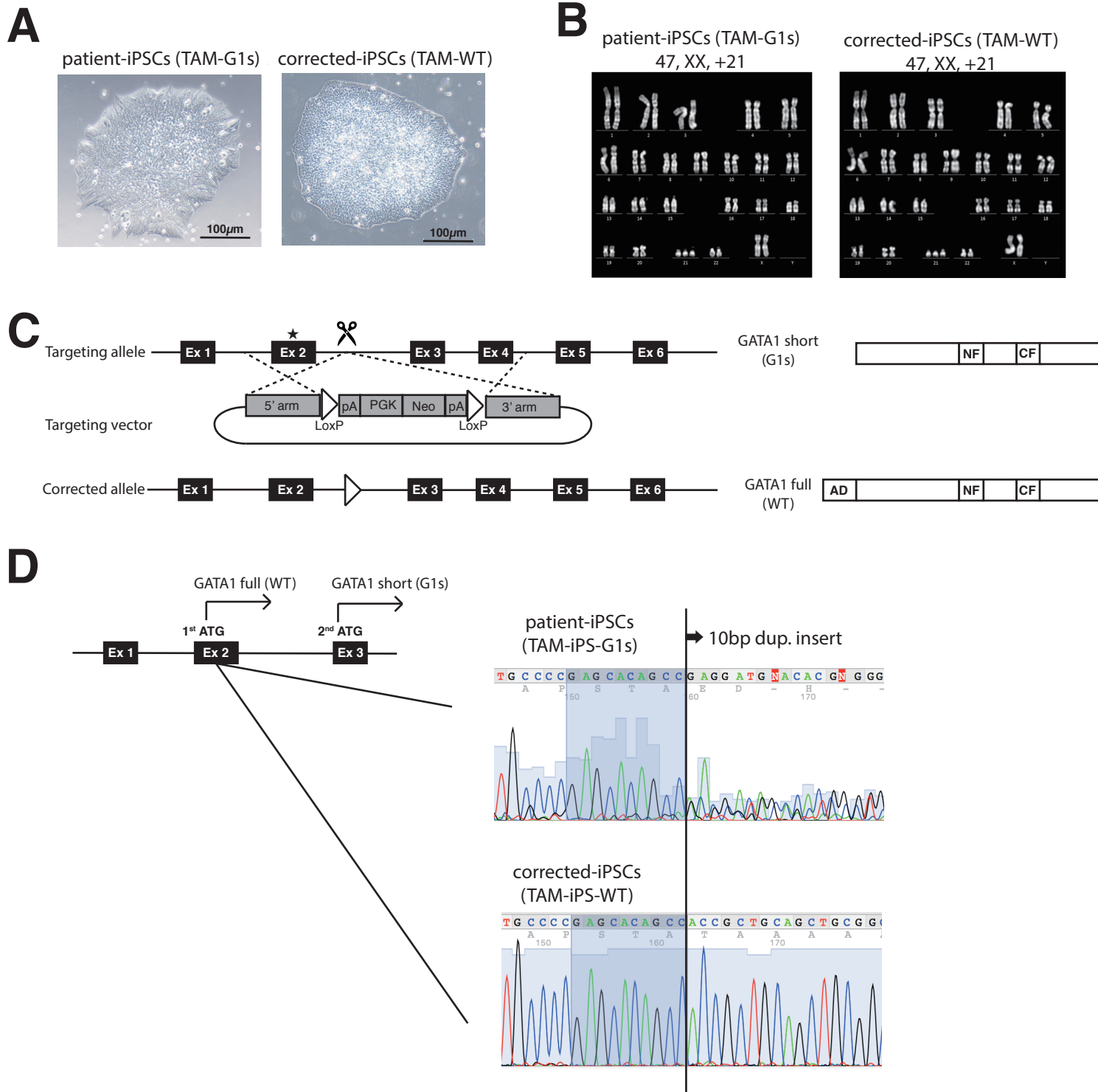
Statistics analyses

Experiments were repeated at least 3 times independently. Values are described as the mean \pm standard deviation (SD). Data were compared with Student's t test and the Pearson correlation using GraphPad Prism (La Jolla, CA, USA). The alpha level of all tests or the p value was set to 0.05.

Supplemental references

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Supplemental Figure 1

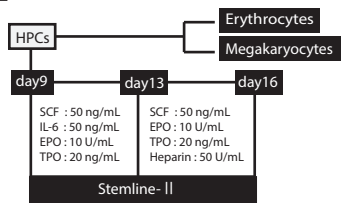


Supplemental Figure 1. Establishment of isogenic iPSC panels from DS-TAM patient primary blood cells

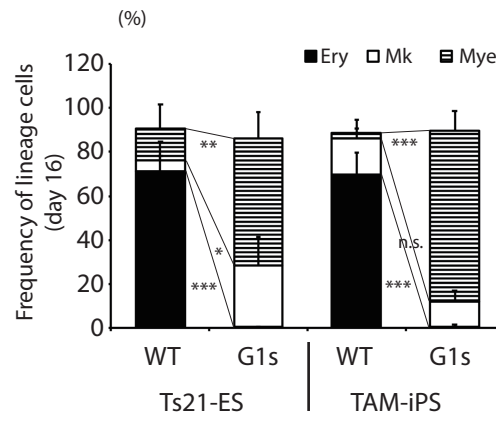
(A) Morphology (phase contrast imaging) and (B) chromosomal analysis (Q-banding) of established iPSCs before (left panel) and after (right panel) gene correction. Scale bars, 100 μm . (C) Schema of the gene correction of *GATA1* mutation using TALEN-mediated gene editing. (D) Representative chromatograph of Sanger sequencing of *GATA1* gene from iPSCs before (upper panel) and after (lower panel) the gene correction.

Supplemental Figure 2

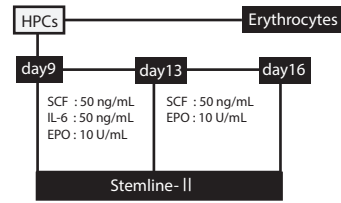
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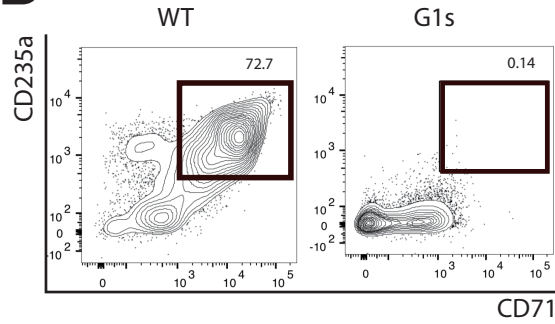
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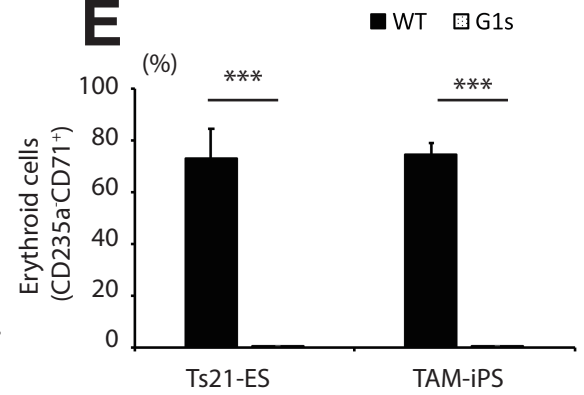
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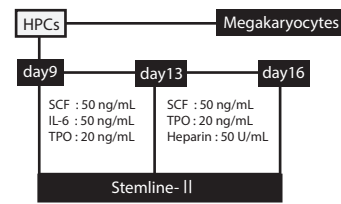
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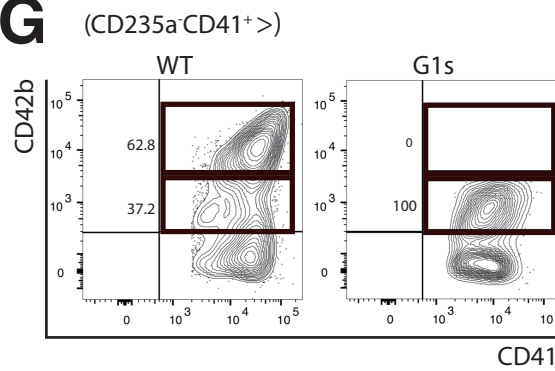
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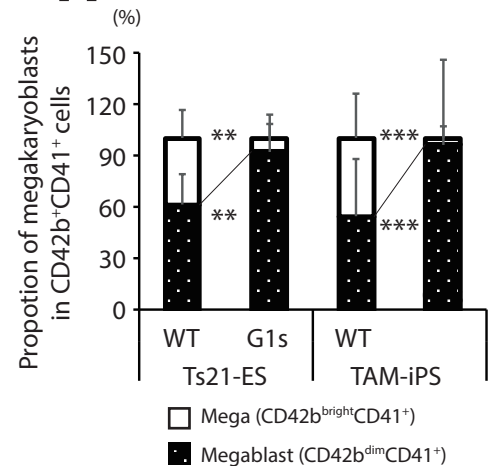
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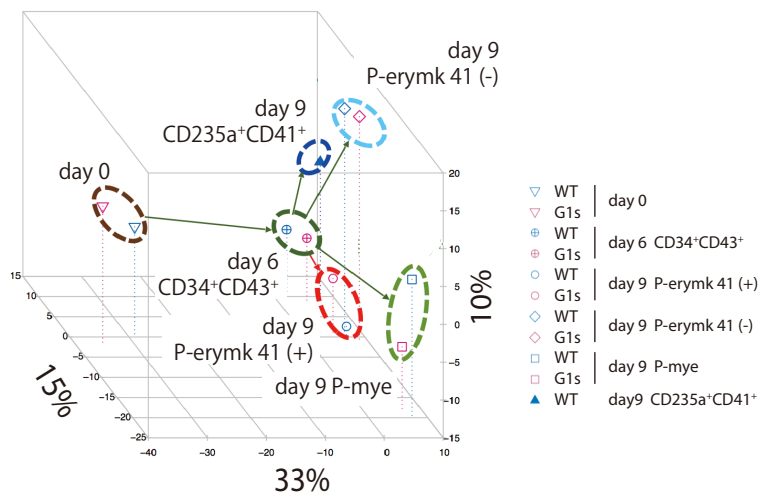


Supplemental Figure 2. Difference in hematopoietic specification between WT and G1s Ts21-PSCs

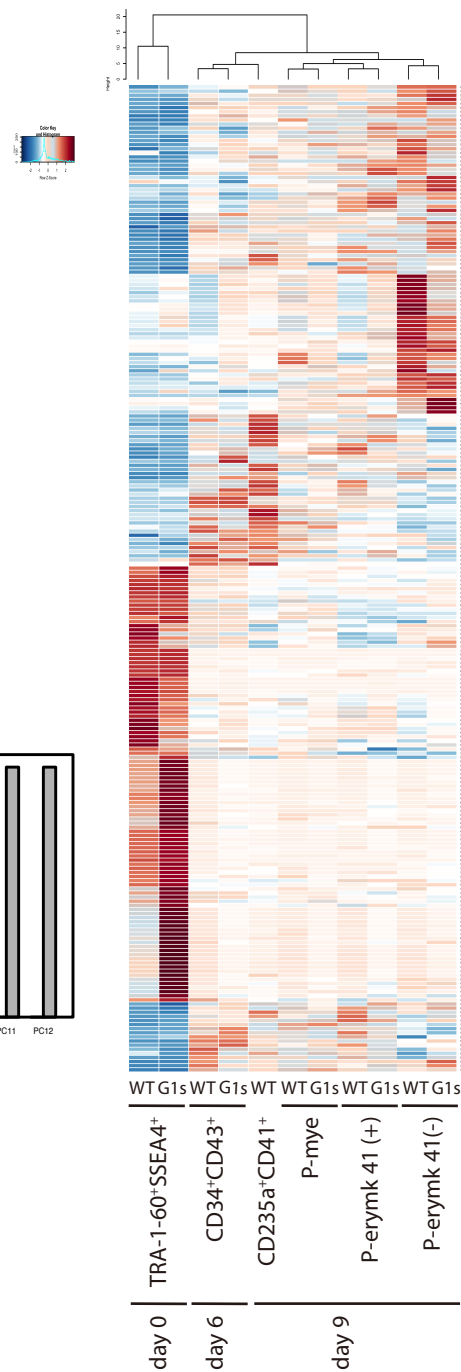
(A) Schematic process for erythroid-megakaryocytic differentiation from hematopoietic progenitor cells. (B) Percentages of erythroid-, megakaryocytic-, and myeloid-lineage commitment according to the differentiation protocol shown in (A). *P* values compare Ery (erythrocytes), Mk (megakaryocytes), and Mye (myeloids) between WT and G1s Ts21-ESCs: < 0.0001, 0.0131, and 0.0016, respectively; and between WT and G1s TAM-iPSCs: < 0.0001, 0.3858, and < 0.0001, respectively. These differences in hematopoiesis were not recovered even under conditions specifically designed to induce erythroid (C-E) or megakaryocytic lineages (F-H). (C) Protocol for erythroid-specific differentiation. (D) Representative FACS panel and (E) percentages of erythroid cells. Dead cells (DAPI positive) were excluded from the analysis. *P* values compare Ery in erythroid lineage-inducing condition between WT and G1s Ts21-ESCs, < 0.0001, and between WT and G1s TAM-iPSCs, < 0.0001. (F) Protocol for megakaryocyte-specific differentiation. (G) Representative FACS panel and (H) percentage of megakaryocytic cells. While megakaryocytic cells from the WT lines contained both CD42b^{dim} (immature) and CD42b^{bright} (more mature) populations, the G1s lines showed only CD42b^{dim} cells, which corresponds to the phenotype seen in the primary blood cells of patients with TAM. Dead cells (DAPI positive) were excluded from the analysis. *P* values compare Mega (megakaryocytes) and Megablast (megakaryoblasts) in megakaryocytic lineage-inducing condition between WT and G1s Ts21-ESCs, 0.0029, and between WT and G1s TAM-iPSCs, 0.0008. Student's t-test. Data are shown as the mean + standard deviation (SD). **P* < .05, ***P* < .01, ****P* < .001; n.s, not significant. (*n* = 4-7).

Supplemental Figure 3

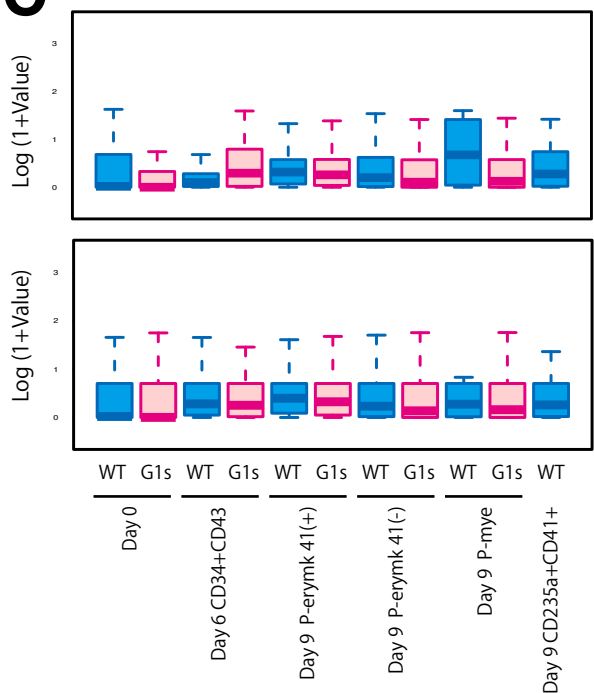
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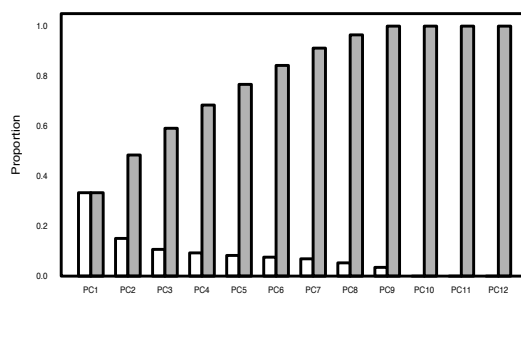
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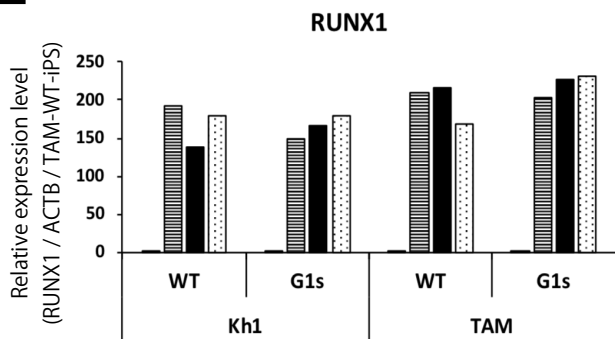
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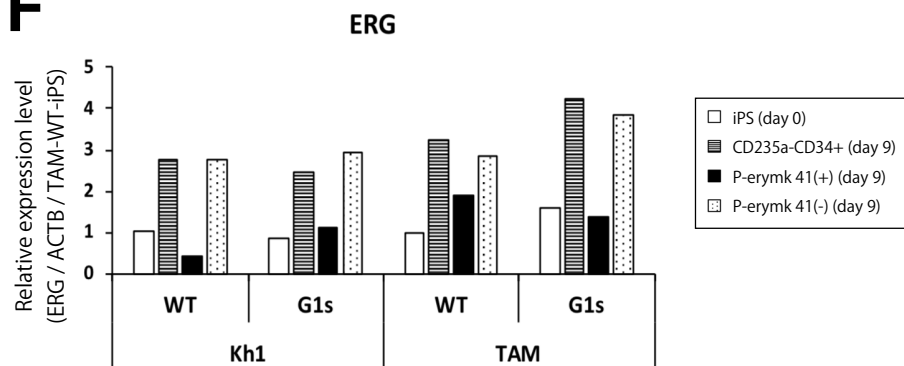
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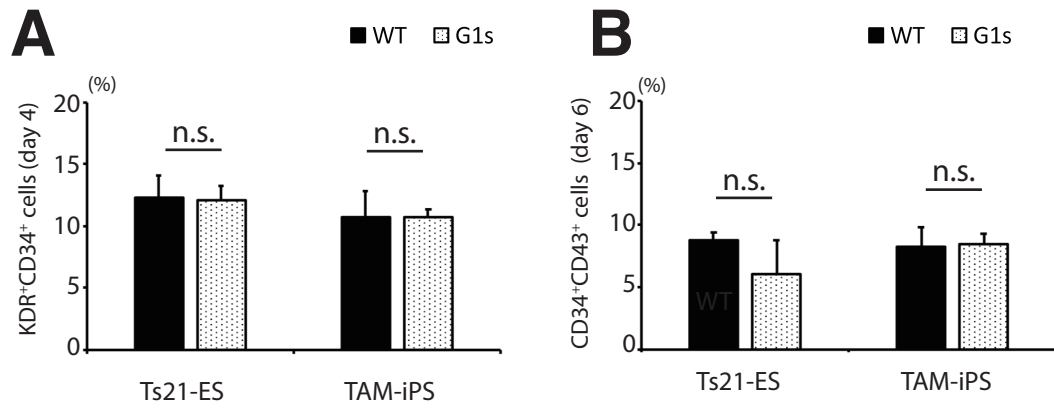
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Supplemental Figure 3. Traced differentiation expression profiles using hematopoiesis-focused PCR array

(A-B) Integrative analysis of the expression profiles using hematopoiesis-focused real-time PCR arrays outlined the transition of gene expression profiles during the differentiation of WT- and G1s-clones and depicted by principal component analysis (A) and a heatmap (B). Data from WT and G1s Ts21-ESCs are shown. (C) Box plots of relative log expressions for unnormalized (upper) and normalized (lower) values. The bottom and top of the boxes indicate the first and third quartiles, respectively; thick lines inside the boxes indicate the medians. (D) Chart of the variance proportion (white) and cumulative variance proportion (gray). (E-F) Relative expression levels of representative hematopoietic genes RUNX1 (E) and ERG (F). These data were analyzed using $\Delta\Delta\text{Ct}$ methods normalized by the ACTB expression level in each sample and gene expression level in WT TAM-iPSCs (day 0).

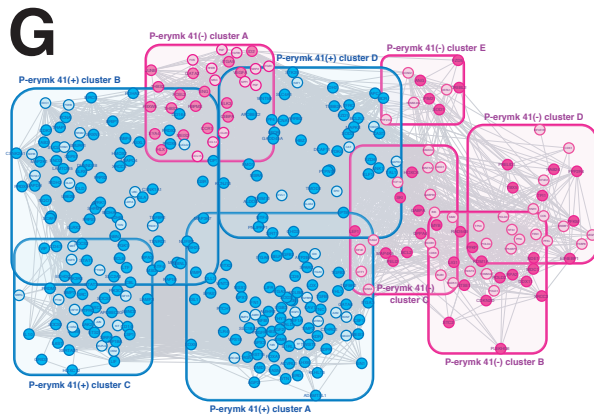
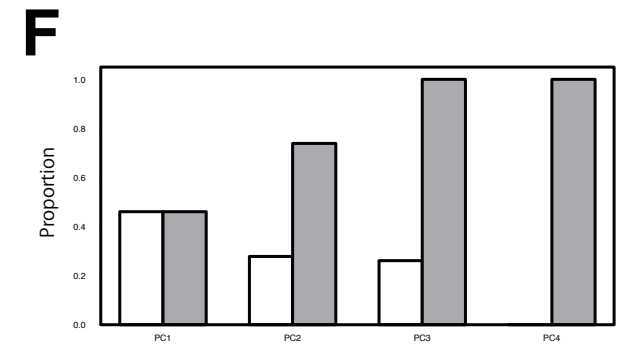
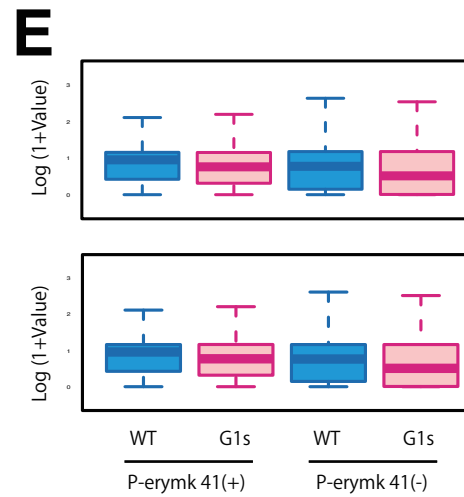
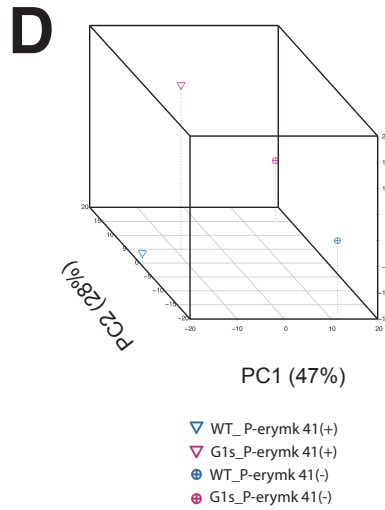
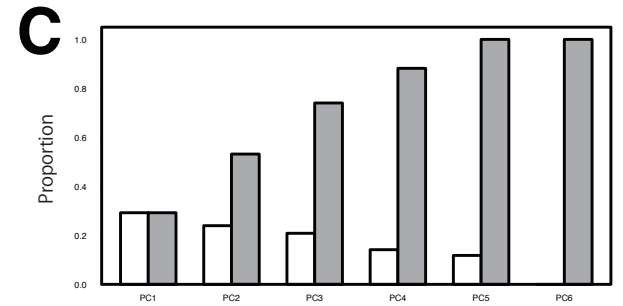
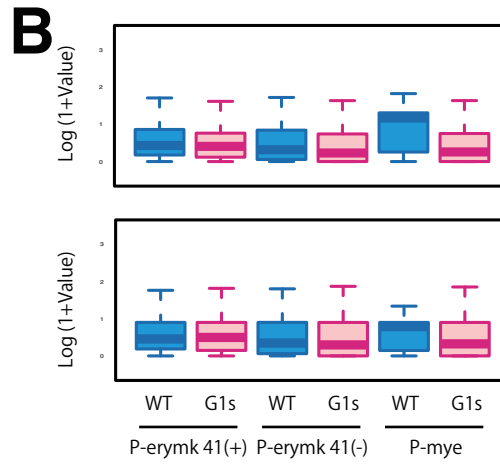
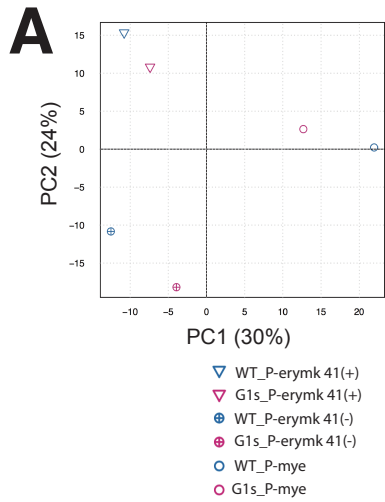
Supplemental Figure 4



Supplemental Figure 4. Mesodermal and early hematopoietic differentiation efficacy

(A-B) Percentages of (A) KDR^+CD34^+ mesodermal (Day 4) and (B) $CD34^+CD43^+$ hematopoietic (Day 6) progenitors.

Supplemental Figure 5



H

Cluster	Lineage specification				GO ID	GO term	Group P-value Corrected with Bonferroni step down
	Pro	Ery	Mk	Mye			
P-erymk 41(+) A					GO: 0010935	regulation of macrophage cytokine production	0.02807078
					GO: 0002719	negative regulation of cytokine production involved in immune response	0.030283099
P-erymk 41(+) B					GO: 0002756	MyD88-independent toll-like receptor signaling pathway	0.006458183
					GO: 0002755	MyD88-dependent toll-like receptor signaling pathway	0.007385797
					GO: 0070423	nucleotide-binding oligomerization domain containing signaling pathway	0.007385797
					GO: 0002223	stimulatory C-type lectin receptor signaling pathway	0.020412957
					GO: 0060218	hematopoietic stem cell differentiation	0.0024106535
P-erymk 41(+) C					GO: 0030888	regulation of B cell proliferation	0.01963206
					GO: 0060334	regulation of interferon-gamma-mediated signaling pathway	0.005517673
					GO: 1903708	positive regulation of hematopoiesis	0.070336045
P-erymk 41(+) D					GO: 0030218	erythrocyte differentiation	9.76476E-06
					GO: 0043249	erythrocyte maturation	0.000428154
					GO: 0061515	myeloid cell development	0.001676536
					GO: 0030219	megakaryocyte differentiation	0.016272944
					GO: 0045639	positive regulation of myeloid cell differentiation	0.017309068

I

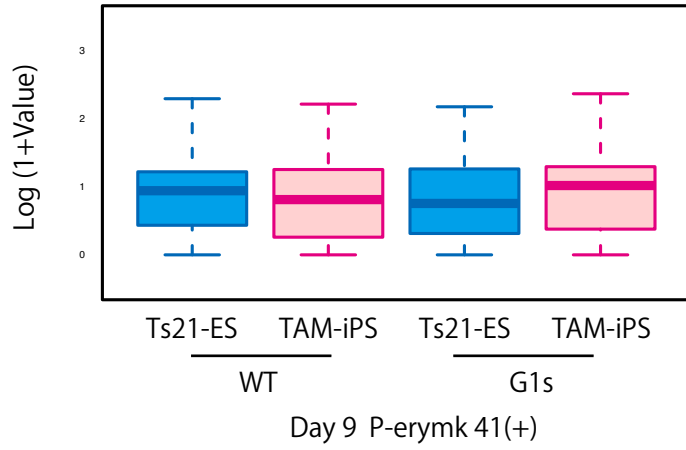
Cluster	Lineage specification				GO ID	GO term	Group P-value Corrected with Bonferroni step down
	Pro	Ery	Mk	Mye			
P-erymk 41(-) A					GO: 0002573	Myeloid leukocyte differentiation	3.36959E-05
					GO: 1903708	positive regulation of hematopoiesis	0.001718853
					GO: 0060319	Primitive erythrocyte differentiation	0.001822897
					GO: 0060754	Positive regulation of mast cell chemotaxis	0.00377464
					GO: 0030225	Macrophage differentiation	0.008782239
					GO: 0030316	Osteoclast differentiation	0.009006904
					GO: 1902105	Regulation of leukocyte differentiation	0.010426746
P-erymk 41(-) C					GO: 0045637	Regulation of myeloid cell differentiation	0.029841227
					GO: 0046632	Alpha-beta T cell differentiation	0.011070477
					GO: 0042093	T-helper cell differentiation	0.024089469
				GO: 1902036	Regulation of hematopoietic stem cell differentiation	0.02759787	

Supplemental Figure 5. *GATA1* mutation impacts P-erymk41(+) (CD34⁺CD43⁺CD235⁻CD11b⁻CD71⁺CD41⁺) subpopulations

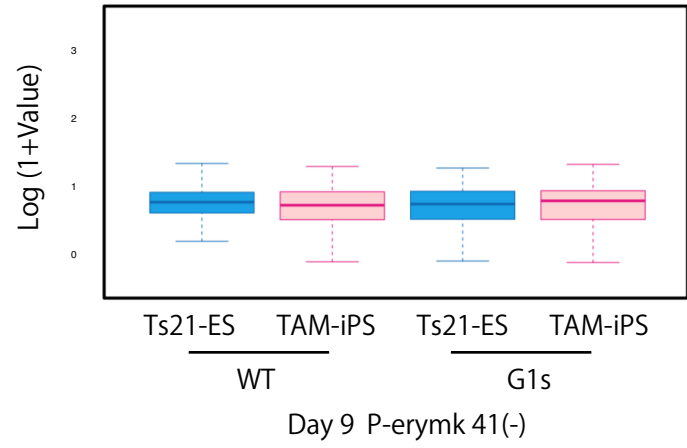
Post-hoc comparison analyses on the expression profiles identified P-erymk41(+) as a responsible population. (A-C) Principal component analysis (PCA) showing differences in the gene expression profiles of P-erymk41(+), P-erymk41(-), and P-mye subpopulations. (A) PCA highlighted differences between CD11b⁺ and both CD11b⁻ populations by mapping them far from each other in the PC1 direction. (B) Box plots of relative log expressions for unnormalized (upper) and normalized (lower) values. The bottom and top of the boxes indicate the first and third quartiles, respectively; thick lines inside the boxes indicate the median. (C) Chart of the variance proportion (white) and cumulative variance proportion (gray). (D) PCA showing gene expression differences in a post-hoc comparison between P-erymk41(+) and P-erymk41(-). (E) Box plots of relative log expressions for unnormalized (upper) and normalized (lower) values. The bottom and top of the boxes indicate the first and third quartiles, respectively; thick lines inside the boxes indicate the median. (F) Chart of the variance proportion (white) and cumulative variance proportion (gray). (G-I) Clustering algorithm for automated functional annotations on a weighted composite functional interaction network based on 261 genes and extracted by the correlation index > 0.58 in a factorial space given by PCA revealed that the CD11b⁻CD71⁺CD41⁺ cells were significantly enriched for pathways related to multi-lineage differentiation, including erythroid, megakaryocytic, and myeloid differentiation, whereas CD11b⁻CD71⁺CD41⁻ cells were enriched in pathways associated with more limited differentiation (P values corrected with Bonferroni step down < 0.03). (G) An expanded map showing the clusters of genes extracted by positive correlations (correlation index > 0.58 in 3D factorial space in the PCA shown in (D)) with P-erymk41+ (blue) and P-erymk (-) (magenta). Closed circles depict genes originally included in the PCR array. Open circles depict genes replenished by GENEMANIA. Each gene is listed in Supplementary Table 2. (H-I) The intracellular pathways specifically enriched in (H) P-erymk41(+) and (I) P-erymk41(-). GO terms with $p < 0.05$ in Bonferroni step down analysis are listed. Closed circles in (H) and (I) show the lineage specifications indicated by the GO terms. The open circle in (I) depicts the indicated relationship with primitive erythrocyte differentiation. There are no significant GO terms for gene sets categorized to P-erymk41(-) clusters B, D or E were enriched.

Supplemental Figure 6

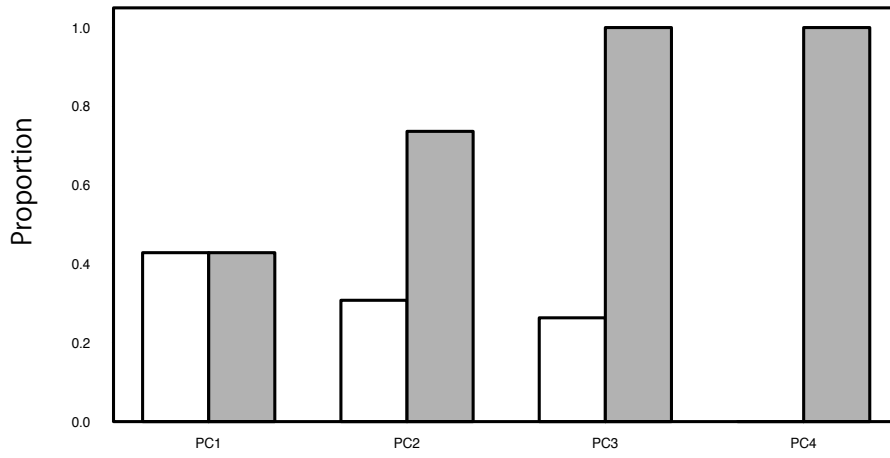
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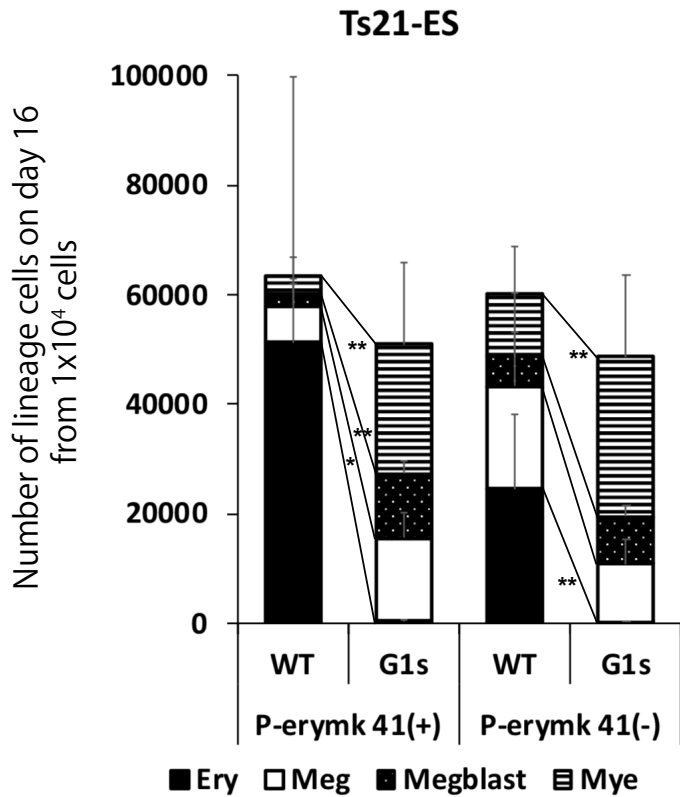


Supplemental Figure 6. Quality check data from principal component analysis of gene expression profiles of P-erymk41(+) derived from WT- and G1s-clones on day 9.

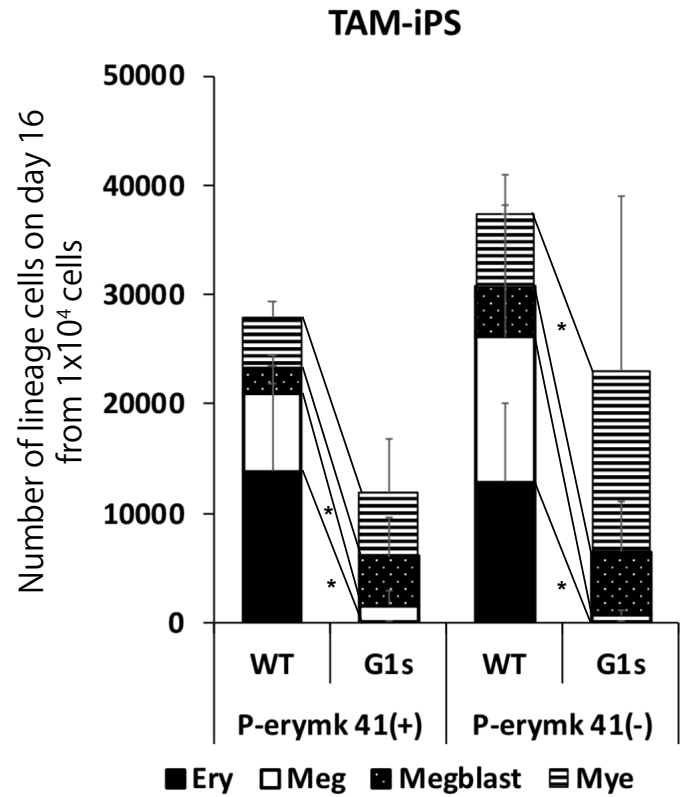
(A-B) Box plots of relative log expressions for unnormalized (A) and normalized (B) values. The bottom and top of the boxes indicate the first and third quartiles, respectively; thick lines inside the boxes indicate the median. (C) Chart of the variance proportion (white) and cumulative variance proportion (gray).

Supplemental Figure 7

A

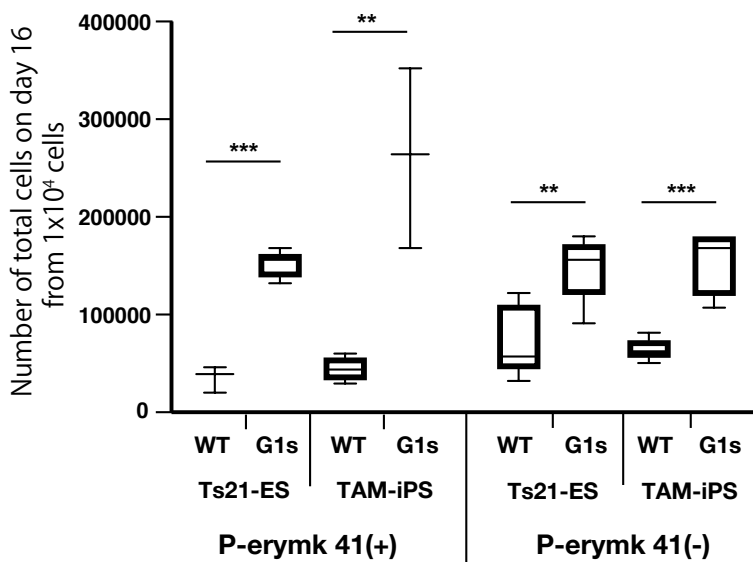


B



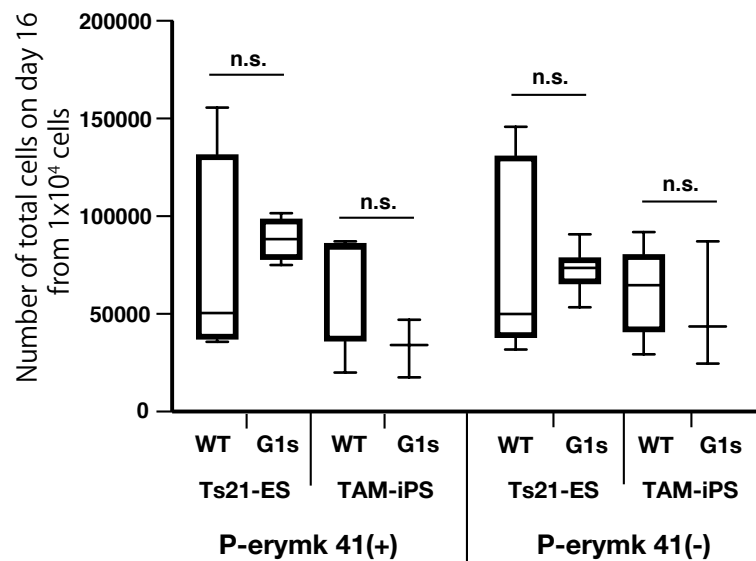
C

Myeloid-specific condition



D

Erythro-megakaryocytic-specific condition



Supplemental Figure 7. Results of the progenitor assay under erythroid-megakaryocytic lineage-specific differentiation condition.

(A-B) Number of lineage cells derived from 1×10^4 sorted (A) Ts21-ES and (B) TAM-iPS of each P-erymk subpopulation under erythro-megakaryocytic lineage-specific differentiation condition. Student's t-test. Data are presented as the mean + SD. * $P < .05$, ** $P < .01$, *** $P < .001$; no mark, not significant. (A) P values compare Ery (erythrocytes), Meg (megakaryocytes), Megablast (magakaryoblasts), and Mye (myeloids) between Ts21-ES-WT and -G1s P-erymk 41(+): 0.0802, 0.0193, 0.0012 and 0.0022; and P-erymk 41 (-): 0.0014, 0.3376, 0.0928 and 0.0034, respectively. (n = 4-6) (B) P values compare Ery, Meg, Megablast, and Mye between TAM-iPS-WT and -G1s P-erymk 41(+): 0.0343, 0.0269, 0.0737 and 0.1912; and P-erymk 41 (-): 0.0245, 0.2098, 0.6173 and 0.0490, respectively. (n = 3-5) (C-D) Total cell number on day 16 derived from 1×10^4 sorted Ts21-ES and TAM-iPS of each P-erymk subpopulation (C) under myeloid lineage-specific differentiation condition and (D) under erythro-megakaryocytic lineage-specific differentiation condition. Multiple comparisons using the Holm-Sidak method. Data are presented as the mean + SD. * $P < .05$, ** $P < .01$, *** $P < .001$; n.s., not significant. (C) P values compare Ts21-ES, TAM-iPS between WT and G1s P-erymk 41(+): <0.001 and 0.004811; and P-erymk 41 (-): 0.002213 and <0.0001 , respectively. (n = 3-7) (D) P values compare Ts21-ES, TAM-iPS between WT and G1s P-erymk 41(+): 0.612962 and 0.141503; and P-erymk 41 (-): 0.950503 and 0.633504, respectively. (n = 3-6)

Assay ID	Gene	Category
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Hs.PT.58v.18759587	B2M	Control
Hs.PT.58v.27737538	GUSB	Control
Hs.PT.39a.22214823	HMBS	Control
Hs.PT.58v.45621572	HPRT1	Control
Hs.PT.39a.22214856.g	RNA18S5	Control
Hs.PT.39a.22214824	RPLP0	Control
Hs.PT.58v.39858774	TBP	Control
Hs.PT.58.26515729	ABCC1	Gene
Hs.PT.58.801316	ABL1	Gene
Hs.PT.58.19167084	ACE	Gene
Hs.PT.56a.4625788	ACO2	Gene
Hs.PT.56a.27778502.g	ACP5	Gene
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Hs.PT.56a.27639918	ADD1	Gene
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Hs.PT.56a.25211580.g	ADM	Gene
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Hs.PT.56a.20260945	ALDOB	Gene
Hs.PT.56a.39340503.g	AMHR2	Gene
Hs.PT.58.40998481	ANAPC2	Gene
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Hs.PT.56a.761468	ANGPT1	Gene
Hs.PT.56a.1015507	ANK1	Gene
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Hs.PT.58.4097385	ANLN	Gene
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Hs.PT.58.3752998	APOBEC3H	Gene
Hs.PT.58.19624591	APOBEC4	Gene
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Hs.PT.58.40418762	ASH2L	Gene
Hs.PT.58.20189842	ASXL1	Gene
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Hs.PT.56a.40556148	BLM	Gene
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Assay ID	Gene	Category
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Hs.PT.58.39941004	BRIP1	Gene
Hs.PT.58.2888747	CACNA1C	Gene
Hs.PT.56a.15372424	CACNB2	Gene
Hs.PT.56a.24396802.g	CALD1	Gene
Hs.PT.56a.2412346	CALM2	Gene
Hs.PT.56a.3619634	CAPN1	Gene
Hs.PT.56a.22796504.g	CAST	Gene
Hs.PT.56a.27208198	CAT	Gene
Hs.PT.56a.40058555.g	CAV1	Gene
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Hs.PT.56a.25419921	CBL	Gene
Hs.PT.58.1474749	CCDC124	Gene
Hs.PT.58.39666925.g	CCL27	Gene
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Hs.PT.56a.39564933	CCNB1	Gene
Hs.PT.58.23013679	CCNB2	Gene
Hs.PT.56a.3857509	CCND1	Gene
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Hs.PT.56a.27776605	CCNE1	Gene
Hs.PT.56a.26264979	CCNF	Gene
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Hs.PT.56a.22530697.gs	CCNT2	Gene
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Hs.PT.58.27365577.g	CD164	Gene
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Hs.PT.58.3602385	CD48	Gene
Hs.PT.56a.38577902	CD80	Gene
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Hs.PT.58.4941264	CREBBP	Gene
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Hs.PT.58.21163170	DHRS3	Gene
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Hs.PT.58.4959174	DLL4	Gene
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Hs.PT.58.39111280	DPPA4	Gene
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Hs.PT.58.1788586	FOXA1	Gene
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Hs.PT.58.2596830	FOXP2	Gene
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Hs.PT.58.26203812.g	FZD2	Gene
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Assay ID	Gene	Category
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Hs.PT.58.19833159	GABPA	Gene
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Hs.PT.58.1794651.gs	GADD45B	Gene
Hs.PT.58.39847030.gs	GADD45G	Gene
Hs.PT.39a.22214836	GAPDH	Gene
Hs.PT.58.21050378	GATA1	Gene
Hs.PT.58.961996	GATA2	Gene
Hs.PT.58.19431110	GATA3	Gene
Hs.PT.58.38396504	GATA6	Gene
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Hs.PT.58.666263.g	GYPA	Gene
Hs.PT.58.23045743.g	H2AFX	Gene
Hs.PT.58.1072699.gs	HBZ	Gene
Hs.PT.58.654007	HDAC4	Gene
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Hs.PT.58.4551980	HDAC7	Gene

Assay ID	Gene	Category
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Hs.PT.58.27232397	HIPK1	Gene
Hs.PT.58.28341041	HK1	Gene
Hs.PT.58.39206966	HK2	Gene
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Hs.PT.58.38958353	ID2	Gene
Hs.PT.58.28216021.g	ID3	Gene
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Assay ID	Gene	Category
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Assay ID	Gene	Category
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Hs.PT.58.14388168	KLF1	Gene
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Hs.PT.58.25540191	LRMP	Gene
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Assay ID	Gene	Category
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Hs.PT.58.38445928	MAML3	Gene
Hs.PT.58.19983600	MAP2K1	Gene
Hs.PT.58.3250219	MAP2K5	Gene
Hs.PT.58.24916350	MAP3K7	Gene
Hs.PT.58.1467068	MAP4K1	Gene
Hs.PT.58.19211033	MAP4K3	Gene
Hs.PT.58.18751097	MAP4K4	Gene
Hs.PT.58.2751839	MAP4K5	Gene
Hs.PT.58.39782850	MAPK1	Gene
Hs.PT.58.25470360	MAPK10	Gene
Hs.PT.58.38819788	MAPK8	Gene
Hs.PT.58.23049618	MAPK9	Gene
Hs.PT.58.841956	MASTL	Gene
Hs.PT.58.28321385	MBNL1	Gene
Hs.PT.58.19109612	MBP	Gene
Hs.PT.58.1431437	MCL1	Gene
Hs.PT.58.46520749	MCM2	Gene
Hs.PT.58.1334606	MCM3	Gene
Hs.PT.58.3215896	MCM4	Gene
Hs.PT.58.45326936	MCM5	Gene
Hs.PT.58.38680806	MDC1	Gene
Hs.PT.58.38439932	MDH2	Gene
Hs.PT.58.40831613	MEIS1	Gene
Hs.PT.58.4390670	MEIS2	Gene
Hs.PT.58.47792779	MGST1	Gene
Hs.PT.58.2859929	MITF	Gene
Hs.PT.58.4553924	MIXL1	Gene
Hs.PT.58.22814824.g	MMP9	Gene
Hs.PT.58.4579399	MNAT1	Gene
Hs.PT.58.680865	MPL	Gene
Hs.PT.58.3471204	MPO	Gene
Hs.PT.58.40957279	MPV17L	Gene

Assay ID	Gene	Category
Hs.PT.58.27516763	MRE11A	Gene
Hs.PT.58.4331480	MSI2	Gene
Hs.PT.58.38848034	MSRA	Gene
Hs.PT.58.2525709	MSX1	Gene
Hs.PT.58.1855833.g	MUS81	Gene
Hs.PT.58.264008	MYB	Gene
Hs.PT.58.2258860	MYH10	Gene
Hs.PT.58.1760411	MYT1	Gene
Hs.PT.58.3438478	NBN	Gene
Hs.PT.58.3970990	NCAM1	Gene
Hs.PT.58.39990207	NCDN	Gene
Hs.PT.58.19204009	NCOA6	Gene
Hs.PT.58.38493496	NDE1	Gene
Hs.PT.58.39625432	NDRG2	Gene
Hs.PT.58.45285779	NDUFA6	Gene
Hs.PT.58.21219212	NDUFB4	Gene
Hs.PT.58.20037448	NDUFS2	Gene
Hs.PT.58.22428357	NEK6	Gene
Hs.PT.58.1801373	NFATC2	Gene
Hs.PT.58.3356525	NFIB	Gene
Hs.PT.58.24326460	NHEJ1	Gene
Hs.PT.58.21333168	NHLH2	Gene
Hs.PT.58.2461055	NKX2-1	Gene
Hs.PT.58.27653648	NLK	Gene
Hs.PT.58.14740388	NOS2	Gene
Hs.PT.58.21447620	NOS3	Gene
Hs.PT.58.23074795	NOTCH1	Gene
Hs.PT.58.28296019	NOTCH2	Gene
Hs.PT.58.15643624	NOTCH4	Gene
Hs.PT.58.24300829	NOX1	Gene
Hs.PT.58.3065606	NOX3	Gene
Hs.PT.58.3866448	NOX4	Gene
Hs.PT.58.40924963	NOX5	Gene
Hs.PT.58.40019160	NPM1	Gene
Hs.PT.58.2697277	NQO1	Gene

Assay ID	Gene	Category
Hs.PT.58.39997829	NR4A1	Gene
Hs.PT.58.45649534	NR5A2	Gene
Hs.PT.58.1271059	NRAS	Gene
Hs.PT.58.25974148	NRP2	Gene
Hs.PT.58.24249783	NTF4	Gene
Hs.PT.58.22560376	NUFIP2	Gene
Hs.PT.58.2015234	NUMA1	Gene
Hs.PT.58.4879244	NUMB	Gene
Hs.PT.58.21180980	OGDH	Gene
Hs.PT.58.40048647.g	OPRM1	Gene
Hs.PT.58.40233450	ORC6	Gene
Hs.PT.58.45339180.g	OSM	Gene
Hs.PT.58.4051301	OSMR	Gene
Hs.PT.58.40853724	PAX2	Gene
Hs.PT.58.3860000	PAX5	Gene
Hs.PT.58.3002797	PAX6	Gene
Hs.PT.58.1092254.g	PCBP4	Gene
Hs.PT.58.2786874	PCF11	Gene
Hs.PT.58.4761611	PCNA	Gene
Hs.PT.58.45699973	PDGFRA	Gene
Hs.PT.58.38803425	PDHA1	Gene
Hs.PT.58.20876171.g	PDHA2	Gene
Hs.PT.58.3078365	PDK1	Gene
Hs.PT.58.40244117	PDK2	Gene
Hs.PT.58.26590302	PDK3	Gene
Hs.PT.58.3853626	PDK4	Gene
Hs.PT.58.46258994	PDLIM1	Gene
Hs.PT.58.25524614	PDP1	Gene
Hs.PT.58.19487865	PECAM1	Gene
Hs.PT.58.21005747	PELI2	Gene
Hs.PT.58.1669823.g	PF4	Gene
Hs.PT.58.19576058	PFKL	Gene
Hs.PT.58.3721	PFKM	Gene
Hs.PT.58.19310435	PFKP	Gene
Hs.PT.58.39519187	PGK1	Gene

Assay ID	Gene	Category
Hs.PT.58.40221999	PGLS	Gene
Hs.PT.58.20312844	PHOX2B	Gene
Hs.PT.58.866326	PIK3CG	Gene
Hs.PT.58.5066731	PIK3R3	Gene
Hs.PT.58.26602866	PILRB	Gene
Hs.PT.58.28069894	PIM1	Gene
Hs.PT.58.25188219	PIM2	Gene
Hs.PT.58.3348936.g	PITX2	Gene
Hs.PT.58.19776078	PLAU	Gene
Hs.PT.58.39400754	PLEKHG6	Gene
Hs.PT.58.15700476	PMS2P5	Gene
Hs.PT.58.3062801.gs	PNKP	Gene
Hs.PT.58.2658525	PNLIPRP1	Gene
Hs.PT.58.25853311.g	PNMT	Gene
Hs.PT.58.39577992	POLD1	Gene
Hs.PT.58.1666726.g	POLD2	Gene
Hs.PT.58.445085	POLD3	Gene
Hs.PT.58.40841836.g	POLD4	Gene
Hs.PT.58.20862405	POLL	Gene
Hs.PT.58.1537882	PPARG	Gene
Hs.PT.58.40982761	PPARGC1A	Gene
Hs.PT.58.25162945	PPP2R4	Gene
Hs.PT.56a.28044743.g	PRDM1	Gene
Hs.PT.58.934660	PRDX1	Gene
Hs.PT.58.4014425.g	PRDX2	Gene
Hs.PT.58.2010183	PRDX4	Gene
Hs.PT.58.338345	PRDX6	Gene
Hs.PT.58.39885312	PRELID1	Gene
Hs.PT.58.14488451	PRKCZ	Gene
Hs.PT.58.345178	PRKDC	Gene
Hs.PT.58.631602.g	PRNP	Gene
Hs.PT.58.19279474	PRPS1	Gene
Hs.PT.58.45647828	PRPS2	Gene
Hs.PT.58.39830063	PTCH1	Gene
Hs.PT.58.39503117	PTPN11	Gene

Assay ID	Gene	Category
Hs.PT.58.14728917	PTPN6	Gene
Hs.PT.58.2558434	PTPRC	Gene
Hs.PT.58.24716816	PVRL2	Gene
Hs.PT.58.40675601.g	RAB24	Gene
Hs.PT.58.4406680	RAB3C	Gene
Hs.PT.58.3272434	RAC1	Gene
Hs.PT.58.14823504	RAD50	Gene
Hs.PT.58.760602	RAD51	Gene
Hs.PT.58.22646548	RAD51B	Gene
Hs.PT.58.40784886	RAD51C	Gene
Hs.PT.58.2390379	RAD52	Gene
Hs.PT.58.27658229	RAD54B	Gene
Hs.PT.58.38442141	RAD54L	Gene
Hs.PT.58.25805985	RAP1B	Gene
Hs.PT.58.364456	RARB	Gene
Hs.PT.58.217934	RBFOX1	Gene
Hs.PT.58.4105281	RBPJ	Gene
Hs.PT.58.28132326	RPMS	Gene
Hs.PT.58.40644908	RGN	Gene
Hs.PT.58.39721574	RGS18	Gene
Hs.PT.56a.40646229.g	RHOB	Gene
Hs.PT.58.2620402	RLIM	Gene
Hs.PT.58.39473758	ROCK1	Gene
Hs.PT.58.19695948	RORA	Gene
Hs.PT.58.19417075	RORC	Gene
Hs.PT.58.20851063	RPA1	Gene
Hs.PT.58.38762774	RPA2	Gene
Hs.PT.58.25693101	RPA3	Gene
Hs.PT.58.25856860.g	RPA4	Gene
Hs.PT.58.20130770	RPE	Gene
Hs.PT.58.2025564	RUNX1	Gene
Hs.PT.56a.20806745	RUNX1T1	Gene
Hs.PT.58.26392294.g	S1PR2	Gene
Hs.PT.58.14833003	SBNO2	Gene
Hs.PT.58.40105692	SCAF4	Gene

Assay ID	Gene	Category
Hs.PT.58.3383192	SCUBE3	Gene
Hs.PT.58.40170798	SDHA	Gene
Hs.PT.58.26657766	SDPR	Gene
Hs.PT.58.15432551	SELE	Gene
Hs.PT.58.41023815	SELP	Gene
Hs.PT.58.2858430	SERTAD1	Gene
Hs.PT.58.3098270	SFXN1	Gene
Hs.PT.58.41102411	SGIP1	Gene
Hs.PT.58.2452854.g	SH2B3	Gene
Hs.PT.58.1201262	SH2D2A	Gene
Hs.PT.58.38373450	SHFM1	Gene
Hs.PT.58.24836645	SHOX2	Gene
Hs.PT.58.27183318	SIRPA	Gene
Hs.PT.58.40382601	SIRT1	Gene
Hs.PT.58.20841096	SIX1	Gene
Hs.PT.58.2548756	SKI	Gene
Hs.PT.58.20087387	SKP2	Gene
Hs.PT.58.24703737	SLAMF1	Gene
Hs.PT.58.2038710	SLC14A1	Gene
Hs.PT.58.27954411	SLC18A2	Gene
Hs.PT.58.39625040	SLC26A7	Gene
Hs.PT.58.19483617	SLC26A9	Gene
Hs.PT.58.39033300	SLC2A3	Gene
Hs.PT.58.3936826	SLC35A2	Gene
Hs.PT.58.19925047	SLC4A1	Gene
Hs.PT.58.39956647	SLC8A3	Gene
Hs.PT.58.20755025	SMAD1	Gene
Hs.PT.58.1353084	SMAD2	Gene
Hs.PT.58.1647675	SMAD3	Gene
Hs.PT.58.472774	SMAD4	Gene
Hs.PT.58.1034954	SMAD5	Gene
Hs.PT.58.27645265.g	SMAD6	Gene
Hs.PT.58.39918935	SMAD7	Gene
Hs.PT.58.18749479	SMAD9	Gene
Hs.PT.58.4506296	SMC1A	Gene

Assay ID	Gene	Category
Hs.PT.58.39588622	SMURF1	Gene
Hs.PT.58.20046277	SMURF2	Gene
Hs.PT.58.39107291.g	SOCS1	Gene
Hs.PT.58.40736839.g	SOCS2	Gene
Hs.PT.58.4303529	SOCS3	Gene
Hs.PT.58.20317581	SOCS5	Gene
Hs.PT.58.40749733	SOD1	Gene
Hs.PT.58.39970926	SOD2	Gene
Hs.PT.58.26108454	SOD3	Gene
Hs.PT.58.24974948.g	SOX4	Gene
Hs.PT.58.22512256	SOX5	Gene
Hs.PT.58.20462363	SPARC	Gene
Hs.PT.58.19735554	SPI1	Gene
Hs.PT.58.4484994	SPN	Gene
Hs.PT.58.19252426	SPP1	Gene
Hs.PT.58.3797199	SPRY4	Gene
Hs.PT.58.1832763	SPTA1	Gene
Hs.PT.58.14624331	SPTB	Gene
Hs.PT.58.19132577	SRC	Gene
Hs.PT.58.3805045	SRD5A3	Gene
Hs.PT.58.26032445.g	SRSF2	Gene
Hs.PT.58.21256932	SRXN1	Gene
Hs.PT.58.2978358	SSBP1	Gene
Hs.PT.58.1013377	STAG3L4	Gene
Hs.PT.58.15049687	STAT1	Gene
Hs.PT.58.14993255	STAT2	Gene
Hs.PT.58.20367494	STAT3	Gene
Hs.PT.58.40907389	STAT4	Gene
Hs.PT.58.19585248	STAT5A	Gene
Hs.PT.58.1687221	STAT5B	Gene
Hs.PT.58.19698584	STAT6	Gene
Hs.PT.58.38362090	STC2	Gene
Hs.PT.58.2768311	STIM2	Gene
Hs.PT.58.1713067	STK25	Gene
Hs.PT.58.20193035	SUCLA2	Gene

Assay ID	Gene	Category
Hs.PT.58.26957310	SUMO1	Gene
Hs.PT.58.724371	SUSD1	Gene
Hs.PT.58.1243965	T	Gene
Hs.PT.58.4566327	TAB2	Gene
Hs.PT.58.40002134	TACC1	Gene
Hs.PT.58.975520	TAL1	Gene
Hs.PT.58.19158678	TBX5	Gene
Hs.PT.58.39386325	TCF12	Gene
Hs.PT.58.15365095	TCF7L2	Gene
Hs.PT.58.19878762	TEK	Gene
Hs.PT.58.27489922	TERT	Gene
Hs.PT.58.26872107	TFEC	Gene
Hs.PT.39a.22214826	TFRC	Gene
Hs.PT.58.22256055	TGFA	Gene
Hs.PT.58.39813975	TGFB1	Gene
Hs.PT.58.24824921	TGFB2	Gene
Hs.PT.58.27186053	TGFB3	Gene
Hs.PT.58.40018323	TGFBI	Gene
Hs.PT.58.39655969	TGFBR1	Gene
Hs.PT.58.3666465	TGFBR2	Gene
Hs.PT.58.19437010	THBS1	Gene
Hs.PT.58.23012211	THBS2	Gene
Hs.PT.58.27473801	THBS3	Gene
Hs.PT.58.21186063	THPO	Gene
Hs.PT.58.22816234	THY1	Gene
Hs.PT.58.470742	TIE1	Gene
Hs.PT.58.27632928	TIMP1	Gene
Hs.PT.58.14780594	TIMP2	Gene
Hs.PT.58.1756331	TIMP3	Gene
Hs.PT.58.21157455	TLN1	Gene
Hs.PT.58.25887499.g	TLR3	Gene
Hs.PT.58.38700156.g	TLR4	Gene
Hs.PT.58.1033703.g	TLX2	Gene
Hs.PT.58.40625927	TMEM180	Gene
Hs.PT.58.1802373	TMOD1	Gene

Assay ID	Gene	Category
Hs.PT.58.2529606	TNC	Gene
Hs.PT.58.45380900	TNF	Gene
Hs.PT.58.23324760	TNFSF11	Gene
Hs.PT.58.24935443	TNFSF13B	Gene
Hs.PT.58.909199.g	TOB1	Gene
Hs.PT.58.14759392	TP53BP1	Gene
Hs.PT.58.39860045.g	TPI1	Gene
Hs.PT.58.39623417.g	TPM3	Gene
Hs.PT.58.19675433	TRAP1	Gene
Hs.PT.58.2459355	TRIM10	Gene
Hs.PT.58.4331913	TSC22D3	Gene
Hs.PT.56a.213661	TSPO	Gene
Hs.PT.58.23073507	TUBB2A	Gene
Hs.PT.58.4545459.g	TUBB2B	Gene
Hs.PT.58.18940950	TWIST1	Gene
Hs.PT.58.24523241	TXN	Gene
Hs.PT.58.1833731	TXNRD1	Gene
Hs.PT.58.45333153	TXNRD2	Gene
Hs.PT.58.24610034	UBE2B	Gene
Hs.PT.58.19793396	VASN	Gene
Hs.PT.58.2346899	VAV1	Gene
Hs.PT.58.20405152	VCAM1	Gene
Hs.PT.58.21234833	VEGFA	Gene
Hs.PT.58.38906895	VIM	Gene
Hs.PT.58.39841194	VIMP	Gene
Hs.PT.58.2333912	VIP	Gene
Hs.PT.58.20957862.g	VNN1	Gene
Hs.PT.58.40010464	VNN2	Gene
Hs.PT.58.22759920.gs	VPS18	Gene
Hs.PT.58.20024588	VRK1	Gene
Hs.PT.58.1205436.g	VTN	Gene
Hs.PT.58.26129052	VWF	Gene
Hs.PT.58.3493460	WBP4	Gene
Hs.PT.58.3793365	WDR81	Gene
Hs.PT.58.1228219	WHSC1L1	Gene

Assay ID	Gene	Category
Hs.PT.58.40456425.g	WNT1	Gene
Hs.PT.58.39365041	WNT10A	Gene
Hs.PT.58.4920373	WNT10B	Gene
Hs.PT.58.40563072	WNT11	Gene
Hs.PT.58.39952122	WNT16	Gene
Hs.PT.58.24288141	WNT2	Gene
Hs.PT.58.26560263	WNT2B	Gene
Hs.PT.58.15704138	WNT3A	Gene
Hs.PT.58.40715343	WNT4	Gene
Hs.PT.58.22221435	WNT5A	Gene
Hs.PT.58.3887638	WNT5B	Gene
Hs.PT.58.2248213	WNT6	Gene
Hs.PT.58.39975999	WNT7A	Gene
Hs.PT.58.125053	WNT7B	Gene
Hs.PT.58.875247	WNT8A	Gene
Hs.PT.58.25267674	WNT8B	Gene
Hs.PT.58.39050185	WNT9A	Gene
Hs.PT.58.40365348	WNT9B	Gene
Hs.PT.58.24479475	XRCC2	Gene
Hs.PT.58.2971406	XRCC3	Gene
Hs.PT.58.38914432	XRCC4	Gene
Hs.PT.58.4426374	XRCC5	Gene
Hs.PT.58.26803952	XRCC6	Gene
Hs.PT.58.28058968	YES1	Gene
Hs.PT.58.2308127.g	ZC3H12A	Gene
Hs.PT.58.2422798	ZFPM1	Gene
Hs.PT.58.20511748	ZMYND8	Gene
Hs.PT.58.1255829	ZW10	Gene

Supplemental Table 1. Gene lists included in the multiplex RT-PCR analysis.

ACTB, B2M, GUSB, HMBS, HPRT1, RNA18S5, RPLP0 and TBP were used as the multiple control genes.

Supplemental Table 2. Values of gene expressions normalized by the multiple control genes.

Gene	Category & Cluster	Origin
A2M	CD41+, Cluster A	GeneMANIA
ACVRL1	CD41+, Cluster A	Array
ADAMTSL1	CD41+, Cluster A	Array
AGP2	CD41+, Cluster A	Array
AMHR2	CD41+, Cluster A	Array
ATP2B4	CD41+, Cluster A	Array
BDNF	CD41+, Cluster A	GeneMANIA
BMP2	CD41+, Cluster A	GeneMANIA
CALD1	CD41+, Cluster A	Array
CAV1	CD41+, Cluster A	Array
CD9	CD41+, Cluster A	Array
CHD2	CD41+, Cluster A	Array
CHST15	CD41+, Cluster A	Array
CSF1	CD41+, Cluster A	Array
CTHRC1	CD41+, Cluster A	Array
DLL1	CD41+, Cluster A	Array
DMD	CD41+, Cluster A	Array
EN1	CD41+, Cluster A	Array
EPAS1	CD41+, Cluster A	Array
ERG	CD41+, Cluster A	Array
F2R	CD41+, Cluster A	GeneMANIA
FHL1	CD41+, Cluster A	Array
FLT1	CD41+, Cluster A	Array
FN1	CD41+, Cluster A	Array
FOXA1	CD41+, Cluster A	Array
GATA6	CD41+, Cluster A	Array
GPX1	CD41+, Cluster A	Array
GPX3	CD41+, Cluster A	Array
HIF1A	CD41+, Cluster A	Array
ID3	CD41+, Cluster A	GeneMANIA
IGF2	CD41+, Cluster A	Array
IL1RL1	CD41+, Cluster A	Array
ISL1	CD41+, Cluster A	Array

Gene	Category & Cluster	Origin
ITGA3	CD41+, Cluster A	Array
ITGA6	CD41+, Cluster A	Array
ITGA7	CD41+, Cluster A	Array
ITGAV	CD41+, Cluster A	GeneMANIA
ITGB3	CD41+, Cluster A	Array
KDR	CD41+, Cluster A	Array
KLHL13	CD41+, Cluster A	Array
LAMB1	CD41+, Cluster A	GeneMANIA
LHX6	CD41+, Cluster A	Array
LIFR	CD41+, Cluster A	Array
LOX	CD41+, Cluster A	Array
MEIS1	CD41+, Cluster A	Array
MSX1	CD41+, Cluster A	Array
NFIB	CD41+, Cluster A	Array
NRCAM	CD41+, Cluster A	GeneMANIA
NTF4	CD41+, Cluster A	Array
NTRK2	CD41+, Cluster A	GeneMANIA
OSMR	CD41+, Cluster A	Array
PDGFRA	CD41+, Cluster A	Array
PDLIM1	CD41+, Cluster A	Array
PITX2	CD41+, Cluster A	Array
PTCH1	CD41+, Cluster A	Array
RGN	CD41+, Cluster A	Array
RHOB	CD41+, Cluster A	Array
SDPR	CD41+, Cluster A	Array
SELP	CD41+, Cluster A	Array
SLC18A2	CD41+, Cluster A	Array
SMAD7	CD41+, Cluster A	GeneMANIA
SOD3	CD41+, Cluster A	Array
SOX5	CD41+, Cluster A	Array
SPARC	CD41+, Cluster A	Array
TF	CD41+, Cluster A	GeneMANIA
TGFB2	CD41+, Cluster A	Array

Gene	Category & Cluster	Origin
TGFB3	CD41+, Cluster A	Array
TGFBI	CD41+, Cluster A	Array
TGFBR2	CD41+, Cluster A	Array
TGFBR3	CD41+, Cluster A	GeneMANIA
THBS1	CD41+, Cluster A	Array
THY1	CD41+, Cluster A	Array
TIMP2	CD41+, Cluster A	Array
TLR4	CD41+, Cluster A	Array
TNC	CD41+, Cluster A	Array
TWIST1	CD41+, Cluster A	Array
VASN	CD41+, Cluster A	Array
VEGFC	CD41+, Cluster A	GeneMANIA
VPS18	CD41+, Cluster A	Array
VTN	CD41+, Cluster A	Array
WNT2	CD41+, Cluster A	Array
ANAPC4	CD41+, Cluster B	Array
ASH2L	CD41+, Cluster B	Array
ASXL1	CD41+, Cluster B	Array
ATF4	CD41+, Cluster B	Array
BIRC5	CD41+, Cluster B	Array
BRIP1	CD41+, Cluster B	Array
CD164	CD41+, Cluster B	Array
CDK7	CD41+, Cluster B	Array
CEP57	CD41+, Cluster B	Array
CSNK1A1	CD41+, Cluster B	Array
CSNK2A1	CD41+, Cluster B	Array
CTCF	CD41+, Cluster B	Array
DENND1B	CD41+, Cluster B	Array
DLAT	CD41+, Cluster B	GeneMANIA
DLD	CD41+, Cluster B	Array
ENO2	CD41+, Cluster B	Array
GAPDH	CD41+, Cluster B	Array
GLA	CD41+, Cluster B	Array

Gene	Category & Cluster	Origin
GLRX	CD41+, Cluster B	Array
GLRX2	CD41+, Cluster B	Array
GSR	CD41+, Cluster B	Array
HMOX2	CD41+, Cluster B	Array
LAMTOR5	CD41+, Cluster B	Array
LDHA	CD41+, Cluster B	GeneMANIA
LDHB	CD41+, Cluster B	Array
MAP2K1	CD41+, Cluster B	Array
MAP3K7	CD41+, Cluster B	Array
MAPK9	CD41+, Cluster B	Array
MBNL1	CD41+, Cluster B	Array
MDH2	CD41+, Cluster B	Array
MPV17L	CD41+, Cluster B	Array
MSI2	CD41+, Cluster B	Array
NBN	CD41+, Cluster B	Array
NQO1	CD41+, Cluster B	Array
NUFIP2	CD41+, Cluster B	Array
ODC1	CD41+, Cluster B	GeneMANIA
PCNA	CD41+, Cluster B	Array
PDHA1	CD41+, Cluster B	GeneMANIA
PDHA2	CD41+, Cluster B	Array
PDHB	CD41+, Cluster B	GeneMANIA
PDK3	CD41+, Cluster B	Array
PDP1	CD41+, Cluster B	Array
PGK1	CD41+, Cluster B	Array
PRDX1	CD41+, Cluster B	GeneMANIA
PRDX2	CD41+, Cluster B	Array
PRDX4	CD41+, Cluster B	Array
PRDX6	CD41+, Cluster B	Array
PRPS1	CD41+, Cluster B	Array
PRPS2	CD41+, Cluster B	Array
RAP1B	CD41+, Cluster B	Array
RPA3	CD41+, Cluster B	Array

Gene	Category & Cluster	Origin
RRM1	CD41+, Cluster B	GeneMANIA
RRM2	CD41+, Cluster B	GeneMANIA
SCAF4	CD41+, Cluster B	Array
SDHA	CD41+, Cluster B	Array
SDHB	CD41+, Cluster B	GeneMANIA
SHFM1	CD41+, Cluster B	Array
SMURF1	CD41+, Cluster B	Array
SRXN1	CD41+, Cluster B	Array
SUCLA2	CD41+, Cluster B	Array
SUCLG1	CD41+, Cluster B	GeneMANIA
TAB2	CD41+, Cluster B	Array
TGFBR1	CD41+, Cluster B	Array
TRAP1	CD41+, Cluster B	Array
TXN	CD41+, Cluster B	GeneMANIA
TXNRD1	CD41+, Cluster B	Array
UBC	CD41+, Cluster B	GeneMANIA
UBE2B	CD41+, Cluster B	Array
VIMP	CD41+, Cluster B	Array
VRK1	CD41+, Cluster B	Array
WBP4	CD41+, Cluster B	Array
XRCC5	CD41+, Cluster B	Array
APOBEC3G	CD41+, Cluster C	Array
BCL6	CD41+, Cluster C	Array
BMP7	CD41+, Cluster C	Array
CBL	CD41+, Cluster C	Array
CD14	CD41+, Cluster C	Array
CD38	CD41+, Cluster C	Array
CD47	CD41+, Cluster C	Array
CSF1R	CD41+, Cluster C	GeneMANIA
ECHDC2	CD41+, Cluster C	Array
EGFR	CD41+, Cluster C	GeneMANIA
ETS2	CD41+, Cluster C	Array
GP1BA	CD41+, Cluster C	Array

Gene	Category & Cluster	Origin
GRID2	CD41+, Cluster C	Array
HOXC12	CD41+, Cluster C	Array
HOXC4	CD41+, Cluster C	Array
IGF1R	CD41+, Cluster C	GeneMANIA
IL10	CD41+, Cluster C	Array
IL10RA	CD41+, Cluster C	GeneMANIA
IL6ST	CD41+, Cluster C	Array
JAK2	CD41+, Cluster C	Array
JUN	CD41+, Cluster C	GeneMANIA
KIT	CD41+, Cluster C	GeneMANIA
LAMP3	CD41+, Cluster C	Array
LIF	CD41+, Cluster C	Array
LSP1	CD41+, Cluster C	Array
LTF	CD41+, Cluster C	Array
MET	CD41+, Cluster C	GeneMANIA
OSM	CD41+, Cluster C	GeneMANIA
PIK3CG	CD41+, Cluster C	Array
PIK3R3	CD41+, Cluster C	Array
PRDM1	CD41+, Cluster C	Array
PTK2	CD41+, Cluster C	GeneMANIA
PTK2B	CD41+, Cluster C	GeneMANIA
PTPRC	CD41+, Cluster C	Array
SBNO2	CD41+, Cluster C	Array
SELPLG	CD41+, Cluster C	GeneMANIA
SERTAD1	CD41+, Cluster C	Array
SIRPA	CD41+, Cluster C	Array
SIRPG	CD41+, Cluster C	GeneMANIA
SLC2A3	CD41+, Cluster C	Array
SOCS1	CD41+, Cluster C	Array
SOCS2	CD41+, Cluster C	Array
SOCS3	CD41+, Cluster C	Array
SRC	CD41+, Cluster C	Array
STAT1	CD41+, Cluster C	Array

Gene	Category & Cluster	Origin
STAT3	CD41+, Cluster C	Array
VIP	CD41+, Cluster C	Array
ALAS2	CD41+, Cluster D	GeneMANIA
ALDOB	CD41+, Cluster D	Array
ANK1	CD41+, Cluster D	Array
APC	CD41+, Cluster D	Array
APOBEC2	CD41+, Cluster D	Array
ARRB2	CD41+, Cluster D	Array
BCL2L1	CD41+, Cluster D	Array
BCR	CD41+, Cluster D	Array
CACNA1C	CD41+, Cluster D	Array
CAST	CD41+, Cluster D	Array
CAT	CD41+, Cluster D	GeneMANIA
DCAF11	CD41+, Cluster D	Array
DVL1	CD41+, Cluster D	GeneMANIA
EPO	CD41+, Cluster D	GeneMANIA
EPOR	CD41+, Cluster D	Array
FMO1	CD41+, Cluster D	Array
FOXO3	CD41+, Cluster D	GeneMANIA
FZD1	CD41+, Cluster D	Array
FZD6	CD41+, Cluster D	Array
GADD45A	CD41+, Cluster D	Array
HBZ	CD41+, Cluster D	Array
IDH2	CD41+, Cluster D	Array
KCNJ13	CD41+, Cluster D	Array
KLF1	CD41+, Cluster D	Array
MAPK3	CD41+, Cluster D	GeneMANIA
MAPK8	CD41+, Cluster D	Array
MSRA	CD41+, Cluster D	Array
PDK4	CD41+, Cluster D	Array
PF4	CD41+, Cluster D	Array
PNLIPRP1	CD41+, Cluster D	Array
PTPN11	CD41+, Cluster D	Array

Gene	Category & Cluster	Origin
RAF1	CD41+, Cluster D	GeneMANIA
SIRT1	CD41+, Cluster D	Array
SLC4A1	CD41+, Cluster D	Array
SOD2	CD41+, Cluster D	Array
SPTB	CD41+, Cluster D	Array
STK25	CD41+, Cluster D	Array
TAL1	CD41+, Cluster D	Array
TFRC	CD41+, Cluster D	Array
TMOD1	CD41+, Cluster D	Array
TRIM10	CD41+, Cluster D	Array
TUBB2A	CD41+, Cluster D	Array
WNT5B	CD41+, Cluster D	Array
WNT6	CD41+, Cluster D	Array
CCL14	CD41-, Cluster A	GeneMANIA
CCL15	CD41-, Cluster A	GeneMANIA
CCL23	CD41-, Cluster A	GeneMANIA
CCR1	CD41-, Cluster A	Array
CEBPA	CD41-, Cluster A	Array
ELK3	CD41-, Cluster A	Array
ENG	CD41-, Cluster A	Array
EYA4	CD41-, Cluster A	Array
FBXW5	CD41-, Cluster A	Array
FOS	CD41-, Cluster A	GeneMANIA
FOSL2	CD41-, Cluster A	Array
GATA2	CD41-, Cluster A	Array
HLX	CD41-, Cluster A	Array
ID2	CD41-, Cluster A	Array
ITGA5	CD41-, Cluster A	Array
ITGB1	CD41-, Cluster A	GeneMANIA
JUNB	CD41-, Cluster A	Array
MEIS2	CD41-, Cluster A	Array
MSC	CD41-, Cluster A	GeneMANIA
NRP2	CD41-, Cluster A	GeneMANIA

Gene	Category & Cluster	Origin
PGF	CD41-, Cluster A	GeneMANIA
RBPMS	CD41-, Cluster A	Array
RHEB	CD41-, Cluster A	GeneMANIA
SPP1	CD41-, Cluster A	GeneMANIA
TGFB1	CD41-, Cluster A	GeneMANIA
THBS2	CD41-, Cluster A	Array
THBS3	CD41-, Cluster A	Array
TNF	CD41-, Cluster A	GeneMANIA
VEGFA	CD41-, Cluster A	Array
BUB1	CD41-, Cluster B	GeneMANIA
CDK6	CD41-, Cluster B	GeneMANIA
CDKN2D	CD41-, Cluster B	Array
DDX11	CD41-, Cluster B	Array
FEN1	CD41-, Cluster B	GeneMANIA
GTSE1	CD41-, Cluster B	Array
H2AFX	CD41-, Cluster B	GeneMANIA
KDM1A	CD41-, Cluster B	Array
LIG1	CD41-, Cluster B	Array
MDC1	CD41-, Cluster B	Array
NDE1	CD41-, Cluster B	Array
PLEKHG6	CD41-, Cluster B	Array
POLD1	CD41-, Cluster B	GeneMANIA
POLD2	CD41-, Cluster B	Array
POLD3	CD41-, Cluster B	GeneMANIA
POLD4	CD41-, Cluster B	GeneMANIA
PPIA	CD41-, Cluster B	GeneMANIA
RAD51C	CD41-, Cluster B	GeneMANIA
RAD54B	CD41-, Cluster B	Array
RPA1	CD41-, Cluster B	GeneMANIA
RPA2	CD41-, Cluster B	Array
STC2	CD41-, Cluster B	Array
TOMM40	CD41-, Cluster B	GeneMANIA
TP53	CD41-, Cluster B	GeneMANIA

Gene	Category & Cluster	Origin
TP53BP1	CD41-, Cluster B	GeneMANIA
XRCC3	CD41-, Cluster B	Array
ADD1	CD41-, Cluster C	GeneMANIA
ATF1	CD41-, Cluster C	GeneMANIA
BCL2	CD41-, Cluster C	Array
BIK	CD41-, Cluster C	GeneMANIA
DPPA4	CD41-, Cluster C	Array
EIF4E	CD41-, Cluster C	GeneMANIA
GABPA	CD41-, Cluster C	Array
GABPB1	CD41-, Cluster C	GeneMANIA
HOXC6	CD41-, Cluster C	Array
LEF1	CD41-, Cluster C	Array
MAP4K5	CD41-, Cluster C	Array
MYB	CD41-, Cluster C	Array
PELI2	CD41-, Cluster C	Array
RAG2	CD41-, Cluster C	GeneMANIA
RCOR1	CD41-, Cluster C	GeneMANIA
SIM2	CD41-, Cluster C	GeneMANIA
SIN3A	CD41-, Cluster C	GeneMANIA
SKI	CD41-, Cluster C	Array
SMAD4	CD41-, Cluster C	GeneMANIA
TCF12	CD41-, Cluster C	GeneMANIA
WNT5A	CD41-, Cluster C	GeneMANIA
CCS	CD41-, Cluster D	GeneMANIA
CDX1	CD41-, Cluster D	GeneMANIA
EIF4E1B	CD41-, Cluster D	GeneMANIA
EIF4E2	CD41-, Cluster D	GeneMANIA
EIF4EBP1	CD41-, Cluster D	Array
FKBP8	CD41-, Cluster D	GeneMANIA
PFKL	CD41-, Cluster D	GeneMANIA
PFKM	CD41-, Cluster D	Array
PFKP	CD41-, Cluster D	Array
PPP2R4	CD41-, Cluster D	Array

Gene	Category & Cluster	Origin
PRELID1	CD41-, Cluster D	Array
RAB24	CD41-, Cluster D	Array
RNH1	CD41-, Cluster D	GeneMANIA
SREBF1	CD41-, Cluster D	GeneMANIA
TBX5	CD41-, Cluster D	Array
TPI1	CD41-, Cluster D	Array
VEGFB	CD41-, Cluster D	GeneMANIA
ANG	CD41-, Cluster E	Array
CCNG1	CD41-, Cluster E	GeneMANIA
CREBL2	CD41-, Cluster E	Array
DERL1	CD41-, Cluster E	GeneMANIA
FZD5	CD41-, Cluster E	Array
PHF21A	CD41-, Cluster E	GeneMANIA
PIM2	CD41-, Cluster E	Array
SOD1	CD41-, Cluster E	Array
TOMM40L	CD41-, Cluster E	GeneMANIA

Supplemental Table 3. Gene sets specifically relevant to P-erymk41(+) and P-erymk41(-) cells.

“Category & Cluster” shows the group to which genes were categorized by PCA and the following ReactomeFI analysis. “Origin” column indicates 261 genes included in the original gene lists (Supplemental Table 1) and 94 genes complemented by the GeneMANIA network algorithm.

Gene	Category & Cluster	Origin
ADAM10	WT, Cluster A	Array
ARNT	WT, Cluster A	Array
CALD1	WT, Cluster A	Array
CEBPD	WT, Cluster A	GeneMANIA
CXCR5	WT, Cluster A	GeneMANIA
DDB2	WT, Cluster A	GeneMANIA
DENND1B	WT, Cluster A	GeneMANIA
DHRS3	WT, Cluster A	GeneMANIA
FHL1	WT, Cluster A	GeneMANIA
HIF1A	WT, Cluster A	GeneMANIA
HK1	WT, Cluster A	GeneMANIA
ICAM4	WT, Cluster A	Array
ID2	WT, Cluster A	GeneMANIA
ID3	WT, Cluster A	Array
ITGA1	WT, Cluster A	GeneMANIA
ITGA5	WT, Cluster A	Array
ITGA7	WT, Cluster A	Array
ITGA9	WT, Cluster A	Array
ITGAL	WT, Cluster A	Array
ITGAV	WT, Cluster A	Array
ITGB1	WT, Cluster A	Array
ITGB3	WT, Cluster A	GeneMANIA
NOTCH2	WT, Cluster A	GeneMANIA
SEMA7A	WT, Cluster A	Array
SRC	WT, Cluster A	Array
TACC1	WT, Cluster A	GeneMANIA
TIE1	WT, Cluster A	GeneMANIA
TNC	WT, Cluster A	GeneMANIA
UBE2B	WT, Cluster A	GeneMANIA
ATF4	WT, Cluster B	Array
BGLAP	WT, Cluster B	Array
CEBPB	WT, Cluster B	GeneMANIA
CREBBP	WT, Cluster B	GeneMANIA

FOXA1	WT, Cluster B	Array
GCG	WT, Cluster B	GeneMANIA
GLI3	WT, Cluster B	Array
HDAC1	WT, Cluster B	GeneMANIA
HDAC2	WT, Cluster B	Array
MAPK10	WT, Cluster B	GeneMANIA
MSI2	WT, Cluster B	Array
RBPJ	WT, Cluster B	GeneMANIA
RUNX1	WT, Cluster B	GeneMANIA
RUNX1T1	WT, Cluster B	Array
RUNX3	WT, Cluster B	GeneMANIA
SMAD5	WT, Cluster B	Array
SON	WT, Cluster B	GeneMANIA
TCF7L2	WT, Cluster B	GeneMANIA
TDRD7	WT, Cluster B	Array
ADD1	WT, Cluster C	GeneMANIA
AHSG	WT, Cluster C	Array
ALAD	WT, Cluster C	Array
BMP6	WT, Cluster C	GeneMANIA
EPO	WT, Cluster C	Array
EPOR	WT, Cluster C	GeneMANIA
GAB2	WT, Cluster C	Array
GHR	WT, Cluster C	GeneMANIA
KLF5	WT, Cluster C	GeneMANIA
PDHX	WT, Cluster C	GeneMANIA
PDK2	WT, Cluster C	Array
SCUBE3	WT, Cluster C	Array
STAT5A	WT, Cluster C	GeneMANIA
STAT5B	WT, Cluster C	GeneMANIA
UBC	WT, Cluster C	Array
VNN1	WT, Cluster C	GeneMANIA
ZBTB16	WT, Cluster C	Array
ASNS	WT, Cluster D	GeneMANIA
BRCA1	WT, Cluster D	GeneMANIA

BRIP1	WT, Cluster D	GeneMANIA
CBFB	WT, Cluster D	GeneMANIA
CKAP5	WT, Cluster D	GeneMANIA
CSNK2A1	WT, Cluster D	GeneMANIA
CSNK2A2	WT, Cluster D	Array
CSNK2B	WT, Cluster D	Array
DDB1	WT, Cluster D	Array
POLR2A	WT, Cluster D	Array
POLR2B	WT, Cluster D	Array
POLR2C	WT, Cluster D	GeneMANIA
POLR2J	WT, Cluster D	Array
RACK1	WT, Cluster D	GeneMANIA
RING1	WT, Cluster D	Array
TUBB2B	WT, Cluster D	GeneMANIA
WNT6	WT, Cluster D	Array
ABL1	MT, Cluster A	GeneMANIA
ARRB2	MT, Cluster A	Array
BNC2	MT, Cluster A	GeneMANIA
CAMK2A	MT, Cluster A	GeneMANIA
CD27	MT, Cluster A	GeneMANIA
CD70	MT, Cluster A	GeneMANIA
CHD7	MT, Cluster A	Array
CSF3	MT, Cluster A	Array
CSF3R	MT, Cluster A	Array
CYTH2	MT, Cluster A	Array
DNM2	MT, Cluster A	Array
DOHH	MT, Cluster A	GeneMANIA
DOK1	MT, Cluster A	GeneMANIA
EGFR	MT, Cluster A	Array
F2R	MT, Cluster A	Array
FES	MT, Cluster A	GeneMANIA
HOXA2	MT, Cluster A	Array
HOXB4	MT, Cluster A	Array
HOXC6	MT, Cluster A	Array

IPCEF1	MT, Cluster A	Array
MAP3K3	MT, Cluster A	Array
MPL	MT, Cluster A	Array
PDK3	MT, Cluster A	Array
PIM1	MT, Cluster A	GeneMANIA
SEMA3A	MT, Cluster A	GeneMANIA
SLAMF1	MT, Cluster A	Array
STAT1	MT, Cluster A	GeneMANIA
TBX5	MT, Cluster A	Array
THBS2	MT, Cluster A	Array
THPO	MT, Cluster A	GeneMANIA
TRIM10	MT, Cluster A	Array
ABCC1	MT, Cluster B	Array
BCL10	MT, Cluster B	Array
CARD11	MT, Cluster B	GeneMANIA
CDK5R1	MT, Cluster B	Array
CDK5R2	MT, Cluster B	GeneMANIA
CDKN1B	MT, Cluster B	GeneMANIA
CSNK1A1	MT, Cluster B	Array
FOS	MT, Cluster B	Array
ID1	MT, Cluster B	Array
JUN	MT, Cluster B	Array
KDM3A	MT, Cluster B	GeneMANIA
MALT1	MT, Cluster B	GeneMANIA
MYB	MT, Cluster B	GeneMANIA
MYF5	MT, Cluster B	GeneMANIA
MYF6	MT, Cluster B	Array
MYT1	MT, Cluster B	Array
NDRG2	MT, Cluster B	GeneMANIA
NFATC2	MT, Cluster B	Array
PCF11	MT, Cluster B	Array
PIAS1	MT, Cluster B	Array
PIN1	MT, Cluster B	GeneMANIA
SIM2	MT, Cluster B	GeneMANIA

SIN3A	MT, Cluster B	Array
SMAD2	MT, Cluster B	GeneMANIA
TAB2	MT, Cluster B	Array
TCF12	MT, Cluster B	GeneMANIA
TERT	MT, Cluster B	GeneMANIA
THBS3	MT, Cluster B	GeneMANIA
WHSC1L1	MT, Cluster B	Array
ANAPC11	MT, Cluster C	GeneMANIA
CCNA2	MT, Cluster C	GeneMANIA
CCNE1	MT, Cluster C	GeneMANIA
CCNH	MT, Cluster C	Array
CDC16	MT, Cluster C	Array
CDC26	MT, Cluster C	GeneMANIA
CDK1	MT, Cluster C	Array
CDK2	MT, Cluster C	GeneMANIA
CDK7	MT, Cluster C	GeneMANIA
CDKN1A	MT, Cluster C	Array
CUL1	MT, Cluster C	GeneMANIA
DLAT	MT, Cluster C	Array
EIF5A	MT, Cluster C	GeneMANIA
GSR	MT, Cluster C	Array
LDHA	MT, Cluster C	GeneMANIA
LDHB	MT, Cluster C	Array
MNAT1	MT, Cluster C	Array
NHP2	MT, Cluster C	GeneMANIA
NPM1	MT, Cluster C	GeneMANIA
SKP1	MT, Cluster C	GeneMANIA
SKP2	MT, Cluster C	Array
CDK5	MT, Cluster D	GeneMANIA
DDX11	MT, Cluster D	GeneMANIA
DHPS	MT, Cluster D	Array
H2AFX	MT, Cluster D	GeneMANIA
MAP2K5	MT, Cluster D	GeneMANIA
MDC1	MT, Cluster D	GeneMANIA

NPM3	MT, Cluster D	GeneMANIA
PKMYT1	MT, Cluster D	GeneMANIA
POLD1	MT, Cluster D	GeneMANIA
POLD2	MT, Cluster D	Array
RAD51	MT, Cluster D	Array
RAD52	MT, Cluster D	GeneMANIA
RPA1	MT, Cluster D	Array
RPA2	MT, Cluster D	Array
RPA3	MT, Cluster D	Array
TP53	MT, Cluster D	Array
TP53BP1	MT, Cluster D	Array
WRAP53	MT, Cluster D	Array
CEP57	MT, Cluster E	Array
DCTN2	MT, Cluster E	Array
DERA	MT, Cluster E	Array
NEK6	MT, Cluster E	Array

Supplemental Table 4. Gene sets specifically relevant to P-erymk41(-) from WT- and G1s-clones.

“Category & Cluster” shows the group to which genes were categorized by PCA and the following ReactomeFI analysis. “Origin” column indicates 103 genes included in the original gene lists (Supplemental Table 1) and 91 genes complemented by the GeneMANIA network algorithm.

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