Induced pluripotent stem cells enable disease modeling and drug screening in calreticulin del52 and ins5 myeloproliferative neoplasms

Running title: CALRdel52/ins5 drive megakaryocytic differentiation

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Supplemental methods

Sequencing and Quantitative real-time PCR, CGH arrays

Genomic DNA was isolated using DNA QiaAmp Kit (Qiagen). Classical PCR was done for *CALRdel52* and *CALRins5* with the primers 5'ACAACTTCCTCATCACCAACG3' and 5'GGCCTCAGTCCAGCCCTG3' and amplicons were run on a 2.5% agarose gel. *CALR* genotyping was also done by fragment sizing using the BigDye Terminator chemistry (ThermoFisher Scientific) on a Genetic Analyzer. *CALR*, *PIK3CD, SETD1B TET2, ASXL1* mutations were sequenced using standard PCR conditions with 100 ng of genomic DNA and specific primers.

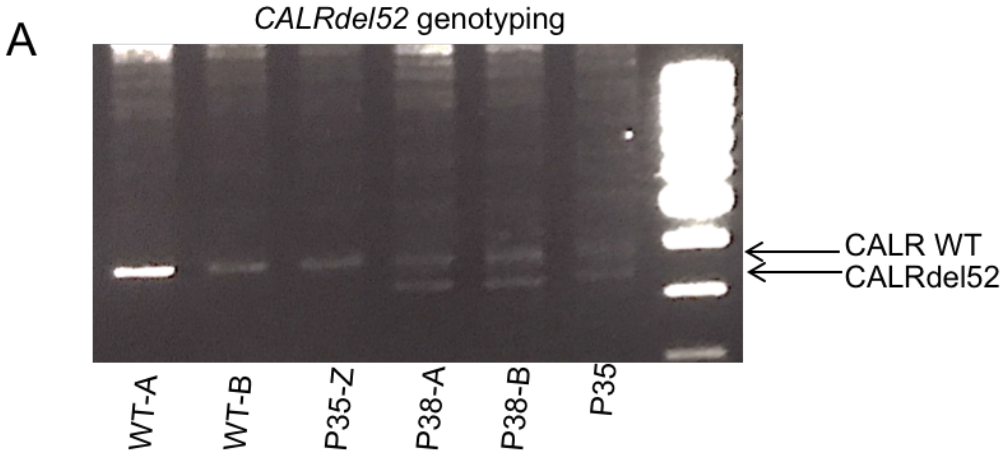
CGH arrays for $CD34^+$ cells and iPSCs were conducted on the human CGH 2x400K platform (G4448A, Agilent Technologies) by hybridization of sample versus matched normal commercially available reference, and hierarchical clustering was performed.

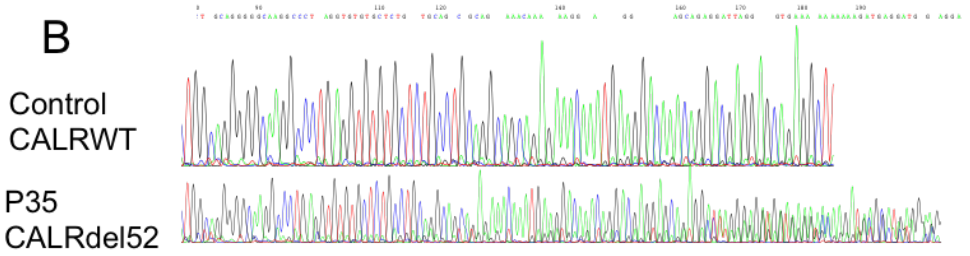
Ploidy analysis in the P34 iPSC was performed by Low-Pass Whole-Genome Sequencing (LP-WGS) (ThermoFisher Scientific). Fragments of genomic DNA (DNAg)

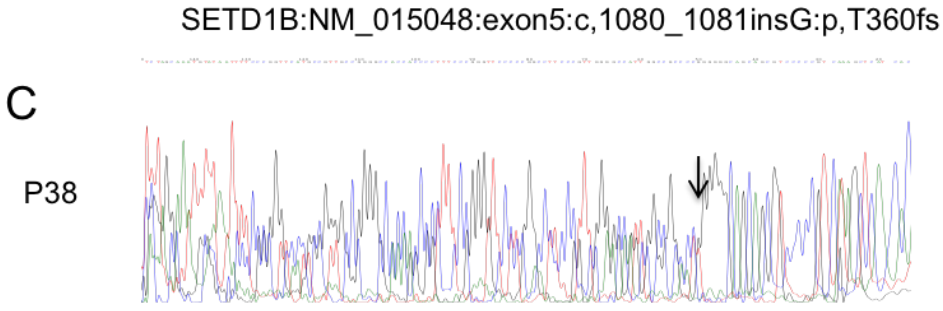
were ligated to sequencing adapters and barcoded using the Ion Xpress™ Plus Fragment Library Kit. The ligated fragments were electrophoretically separated in agarose gels (2%). Library was amplified by PCR using universal primers. After, sequencing templates were enriched by emulsion PCR using the Ion PGM™ Hi-Q™ View OT2 chemistry on an Ion OneTouch™ 2 System. Enriched templates were mixed into a single pool and applied to Ion 316™ v2 Chip. Sequencing was carried out on Ion PGM System using the Ion PGM™ Hi-Q™ View chemistry and analyzed by Ion Reporter software. The LP-WGS was analyzed by the Low-Pass Whole-Genome aneuploidy r.0v5.0 automated analysis workflow.

The Whole Exome Sequencing (WES) was performed using HiSeq2000 after capture with Agilent kit (SureSelect v4). We analyzed the results by comparing $CD3^+$ non-tumoral cells to $CD34^+$ cells from patients.

Supplemental figures:







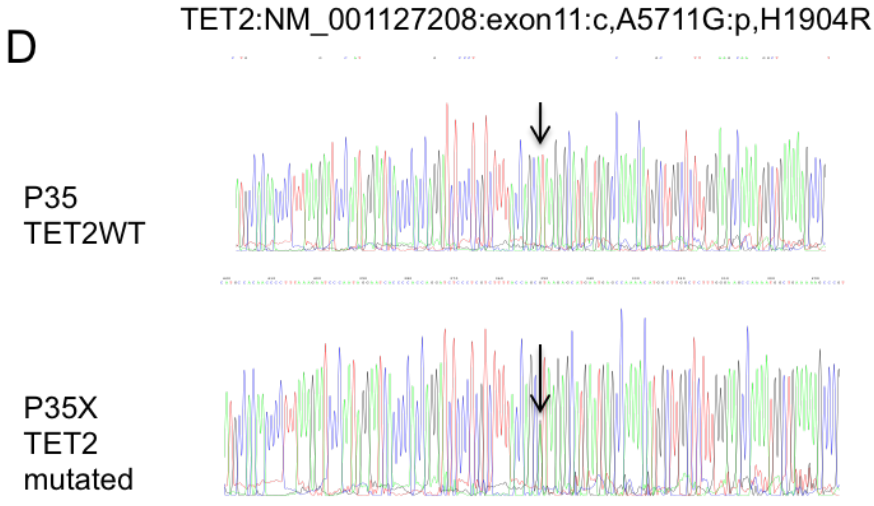


Figure S1: Molecular characterization of *CALRdel52* iPSCs. Genomic DNA from P35, P38 or WT iPSC were prepared. (A) *CALRdel52* genotyping was performed by PCR and amplicons were run on a 2.5% agarose gel. P35-Z is an iPSC obtained from patient #35 but that was WT for *CALR*. (B) *CALR* gene was also sequenced and electropherogram are presented for WT iPSC or P35-iPSC (*CALRdel52*). (C) *SETD1B* exon5:c,1080_1081insG mutation were sequenced for P38-A and P38-B iPSC and electropherogram are shown. (D) *TET2* exon11:c, A5711G mutation was sequenced for P35 iPSC and for another *TET2*-positive iPSC and electropherograms are shown.

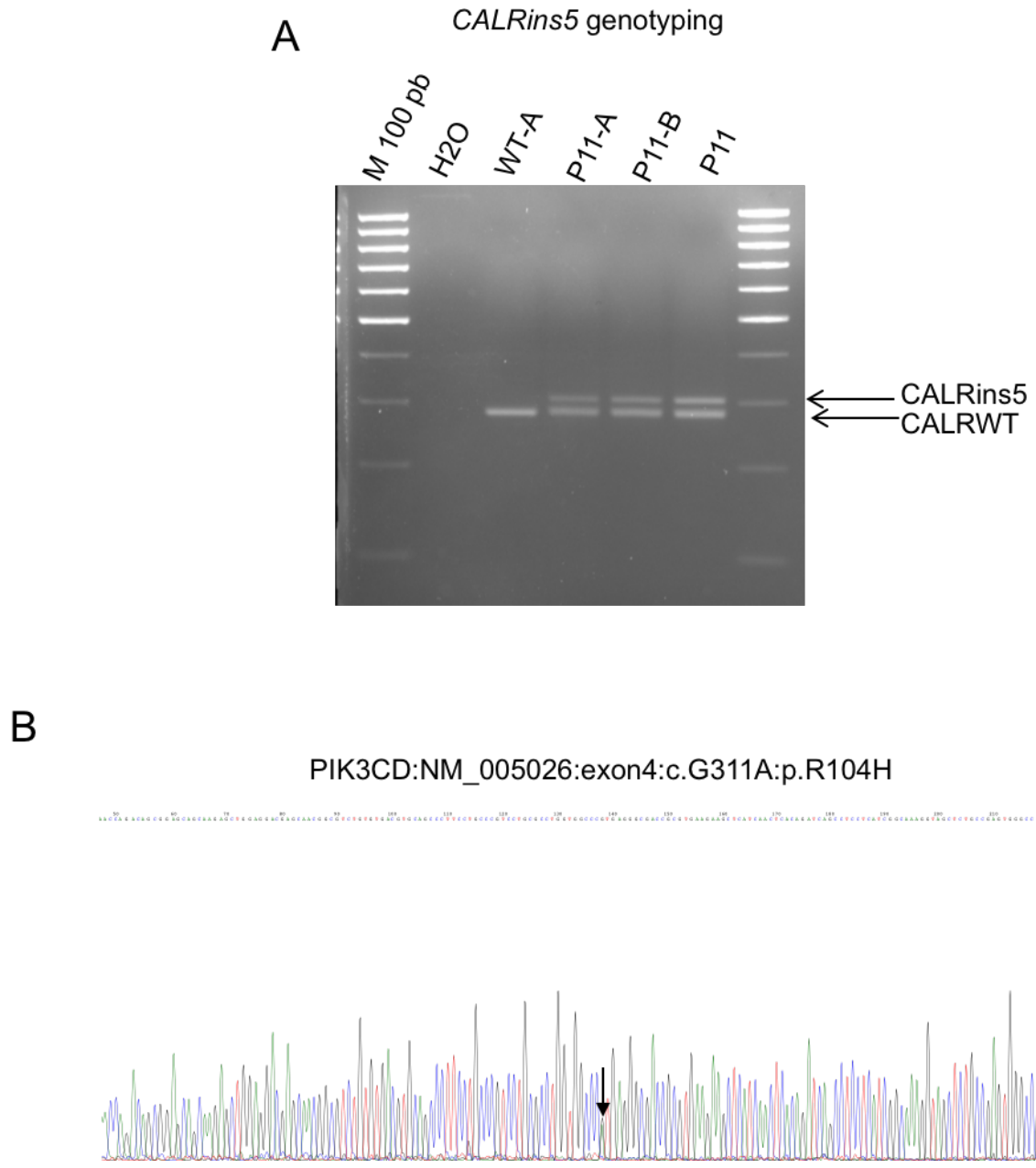


Figure S2: Molecular characterization of *CALRins5* iPSC from #11. Genomic DNA from WT and P11 iPSCs were prepared. (A) *CALRins5* genotyping was performed by PCR and amplicons were run on a 2.5% agarose gel. P11-A and P11-B are iPSC obtained from patient 11 (#11). (B) *PIK3CD* exon4:c.G311A mutation was sequenced for P11 iPSC and electropherogram is shown.

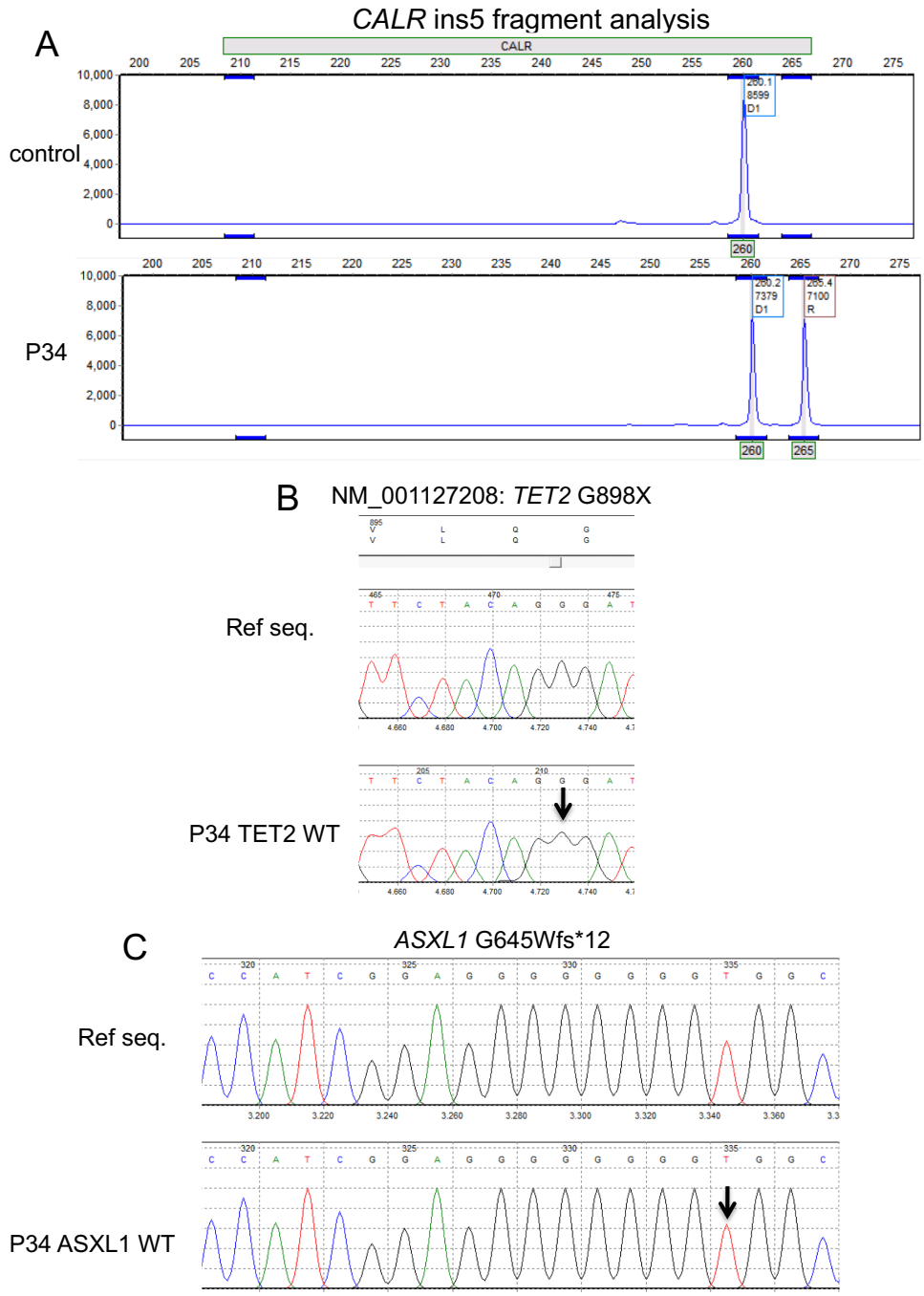


Figure S3: Molecular characterization of *CALRins5* iPSC from #34. Genomic DNA from WT and P34 iPSCs were prepared. (A) *CALRins5* genotyping was performed by fragment sizing using the BigDye Terminator chemistry (Life Technologies) on a Genetic Analyser. (B) *TET2 G898X* and *ASXL1* G645Wfs*12 mutations were sequenced for P11 iPSCs and electropherogram are shown.

WT-A

WT-B

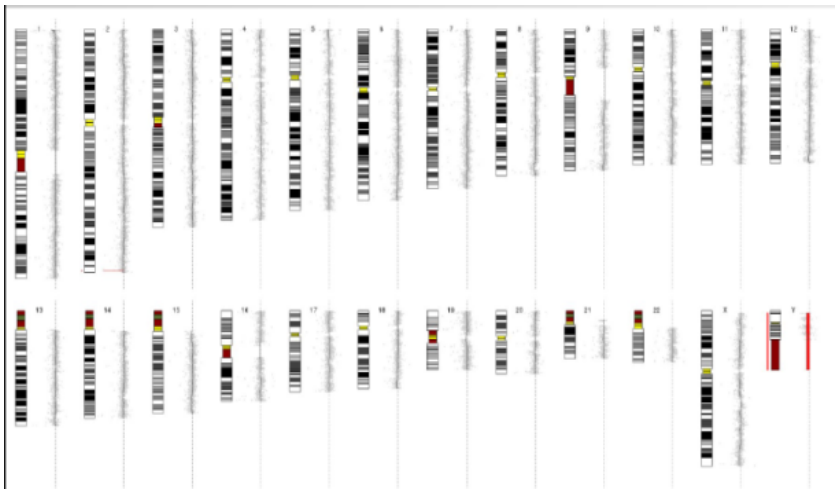


Figure S4: Karyotypes were evaluated for WT-A and WT-B.

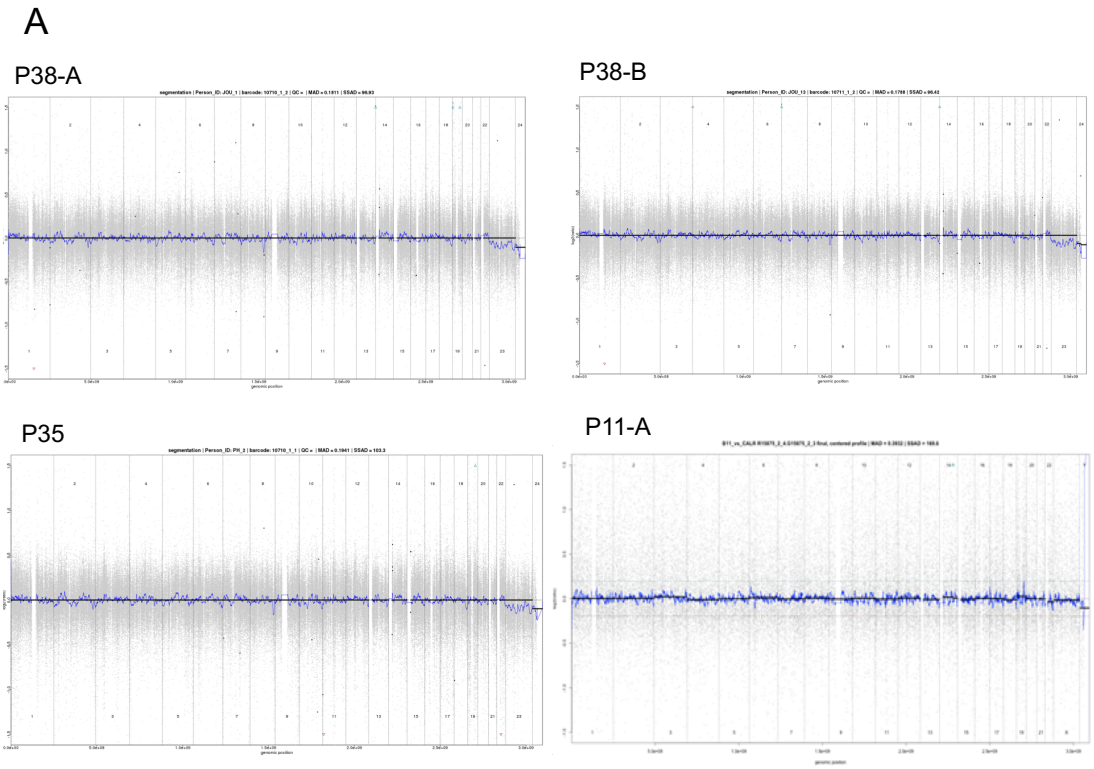


Figure S5: Molecular characterization of *CALRdel52* and *CALRins5* iPSC. (A) CGH arrays for iPSC were conducted on the human CGH 2x400K by hybridization of sample *versus* matched normal commercially available reference for P38-A, P38-B, P35 and P11. (B) Ploidy analysis for P34 iPSC was performed by low-pass whole-genome sequencing.

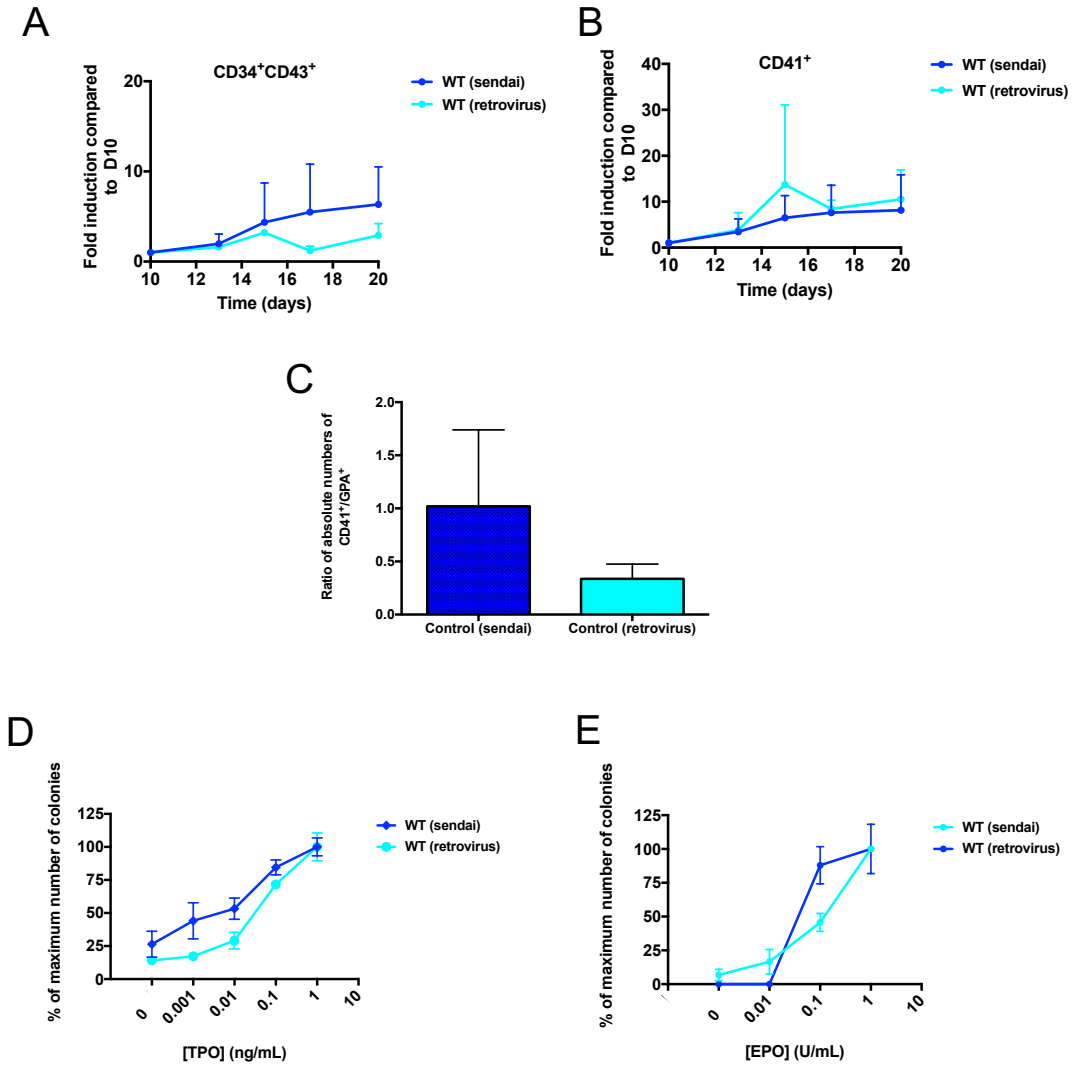


Figure S6: Hematopoietic phenotypes observed with the 2 iPS clones WT generated by Sendai were compared to 2 iPS clones WT generated by retrovirus. (A, B, C) Kinetics of hematopoietic differentiation from day 10 to day 20 of control iPS generated with Sendai and control iPSC generated with retroviral vectors. (A) Results for $CD34^+CD43^+$ cells and (B) $CD41^+$ cells are expressed as fold induction compared to day 10 (mean ± SEM, Control (Sendai) n=10; Control (retrovirus) n=3) (C) $CD41^+/GPA^+$ ratios of absolute number of cells in Control (Sendai) and Control (retrovirus) at day 12 (mean ± SEM, Control (Sendai) n=3; Control (retrovirus) n=3). (D) Sensitivity of CFU-MK to TPO. (E) Sensitivity of EryP to EPO (mean ± SEM, Control (Sendai): n=7; Control (retrovirus) n=4)

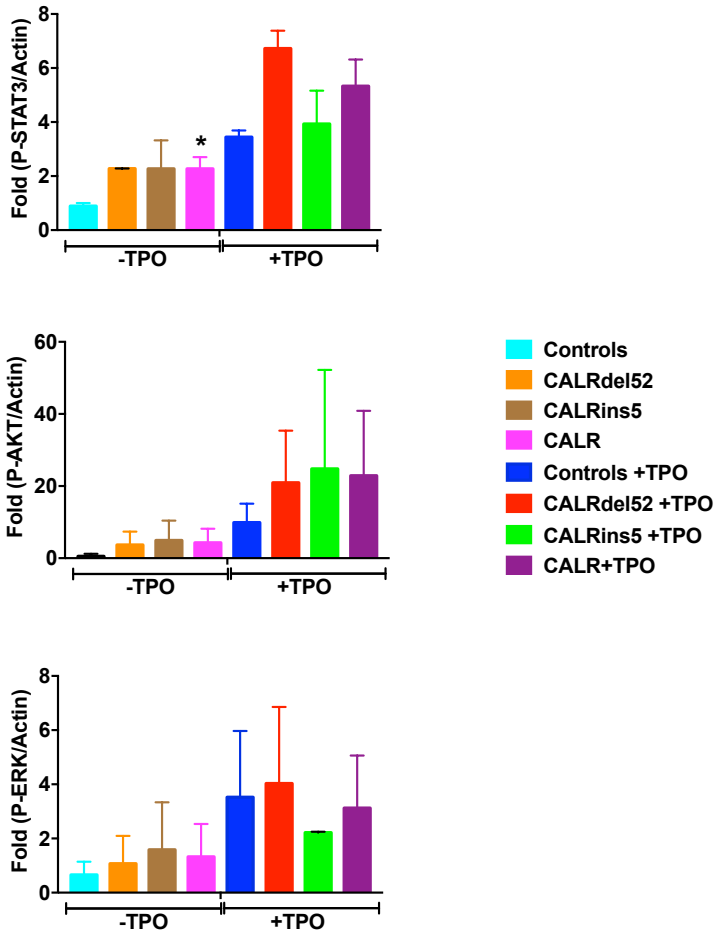


Figure S7. CALRdel52/ins5 induce a constitutive activation of the JAK2/STAT pathway in megakaryoblasts. Day 12-megakaryoblasts ($CD41^{+}$) from all the iPSCs were sorted, cytokine-deprived for 24 hours and then restimulated or not with 20 ng/mL of TPO and subjected to western blot analysis using specific antibodies. Fold increase in P-STAT3, P-AKT and P-ERK were calculated. Experiment were performed with the 2 *CALRdel52* and 2 *CALRins5* iPSC. CALR represents CALRdel52+CALRins5. *p<0.05 Kruskal-Wallis, Multi comparison test.