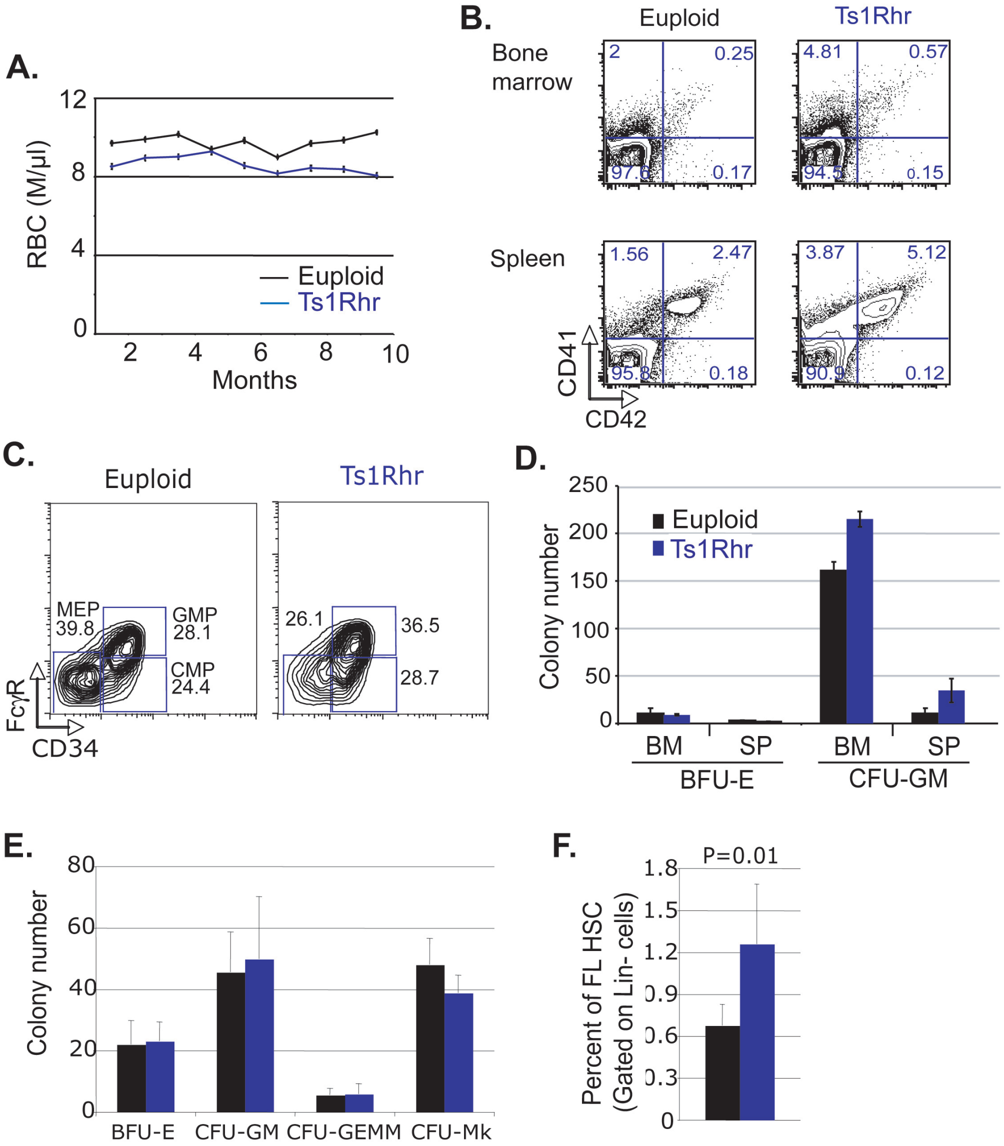
Supplemental table 1:
List of primers used to assess trisomic gene expression.

GENE NAME	FORWARD PRIMER	REVERSE PRIMER
Human genes		
STCH	GGATGTTTCTAACCCGAGCA	TCTTTCCTAGAGGGCACGAA
SAMSN1	CAGCTGCTGAAAACTTCCT	GTCCCTGGGCAGTCATCT
GABPa	CCTGAACTGGTTGCACAGAA	ACAAATCATGTCCCCATCGT
BACH1	TGCCCATATGCTTGTGTCAT	TGTGCATTGAATGGCAGTTT
SOD1	TGGCCGATGTGTCTATTGAA	CACCTTTGCCCAAGTCATCT
SYNJ1	GCAGGCATTTCTTGGCTTAG	TGAAAGCGAGTCACCAACTG
C21ORF66	TCGGAGTTTTCAAGCCATTC	CTGCCATCTTACCGGTTTGT
IL10Rb	GCTGTGGTGCGTTTACAAGA	TGGCCCAAAAACTCTTTCAG
IFNAR1	TTGGGAAAACACTTCAAATGC	GTGCTCTGGCTTTCACACAA
IFNGR2	GTCGGGCATTTAAGCAACAT	AATGTTCCCACGGAGATCAG
DSCR1	AGGCTCCAGCTGCATAAGAC	GGTCGCATCTTCCACTTGTT
SETD4	CTTAGCGCCTGCTTGTTTTC	GAATCACTGTGTCCGTGGTG
CBR3	ATGATCCAATGCCCTTTGAC	ACTGCAAACTACTGATATTCACC
DOPEY2	GTACATGCTGGGGACCAATC	GGATGGCTCTCTCATTGGAA
MORC3	ACATGGGTGTTGGAGTGGTT	TCATTCAGCTTTTCTCCTAGTGC
CHAF1B	ACGGACACTCCACCAAGTTC	CGTGCCTCCTTTGTTTTCAT
HLCS	GCCTCAGCTGTGACATGAAA	AGTCCACATGTTTCCCAAGC
DSCR5	GTGTGGGCCTTTATTCCTGA	GGATGGAGTCGAGTGGAGAG
TTC3	TGCAGGCGATGTAACAATTC	AAGCAAATTGCAGTCTTCCA
DSCR3	TTCCCTAGGCTGTTCACCTG	GTTCTCCGTGATGAGGTGGT
DYRK1A	AGTTCTGGGTATTCCACCTGCTCA	TGAAGTTTACGGGTTCCTGGTGGT
ERG	GCTGCTCAACCATCTCCTTC	ACAGGAGCTCCAGGAGGAAC
ETS2	TGGAGACGGATGGGAGTTTA	CGACGTCTTGTGGATGATGT
DSCR2	AATCCAATCCCTCGGTTTTT	TGCATGTTCTTCCTTGGACA
BRWD1	CAGCAGCAGCAAGATCAGAG	CTTGTCCACTACGACGCAGA
HMGN1	AAGGAAGAGCCCAAGAGGAG	TCCCCTTTTCCCTTTTGTTT
WRB	GAAAGCTCGGACAGCTCAAT	ATCCATTTACTCGGCACGAC
LCA5L	GGAAAAGGATCGTGAGCTTG	GGGTTCCCTGGTGTCTCATA
SH3BGR	TCAAAATGGGATTCCTTTGC	TGCCTCAGTTTCTCCACCTT
BACE2	GCGGCTACTACCTGGAGATG	TATGTAGGAGTGCGGGGTTC
MX1	ACCACAGAGGCTCTCAGCAT	CTTCAGGTGGAACACGAGGT
FAM3B	TCGCAGAGCTCATTCCAGAT	GGGAGTCCAGTGGTCACATT
GATA1	TTAGCCACCTCATGCCTT	GAGACTTGGGTTGTCCAG
GATA2	ATCAGCCCAAGCGAAGACT	CATGGTCAGTGGCCTGTTAA
CD42	ACCTGACCAAAGGCTTCACA	CAGCTGGTGACTAGGGAAGG
GAPDH	GAAGGTGAAGGTCGGAGT	GAAGATGGTGATGGGATT
Murine genes		
Dyrk1a	GCAGGTGTCTGCCTTACCAT	AGGAGCAGTTGCTGGATCAC
Chaf1b	TCTTTCCCATTTGGCTATGTG	GGAAGGTCACAAATGTGCAA
Hlcs	GACAGGTTTCAGGACCAAGG	GAGTCATCAAGGCCCACAAT
Morc3	CTGCAGGGAGGAAAGGGTAT	TGTCCACGTATGATGATCTGC
Hmgn1	CTGACCAGCAAAACCACAGAG	ACCACTGACAGACGTGATGG
Gapdh	TGCACCACCAACTGCTTAG	GATGCAGGGATGATGTTC

Supplemental table 2:
shRNA constructs used for the functional screening in CMY cells.

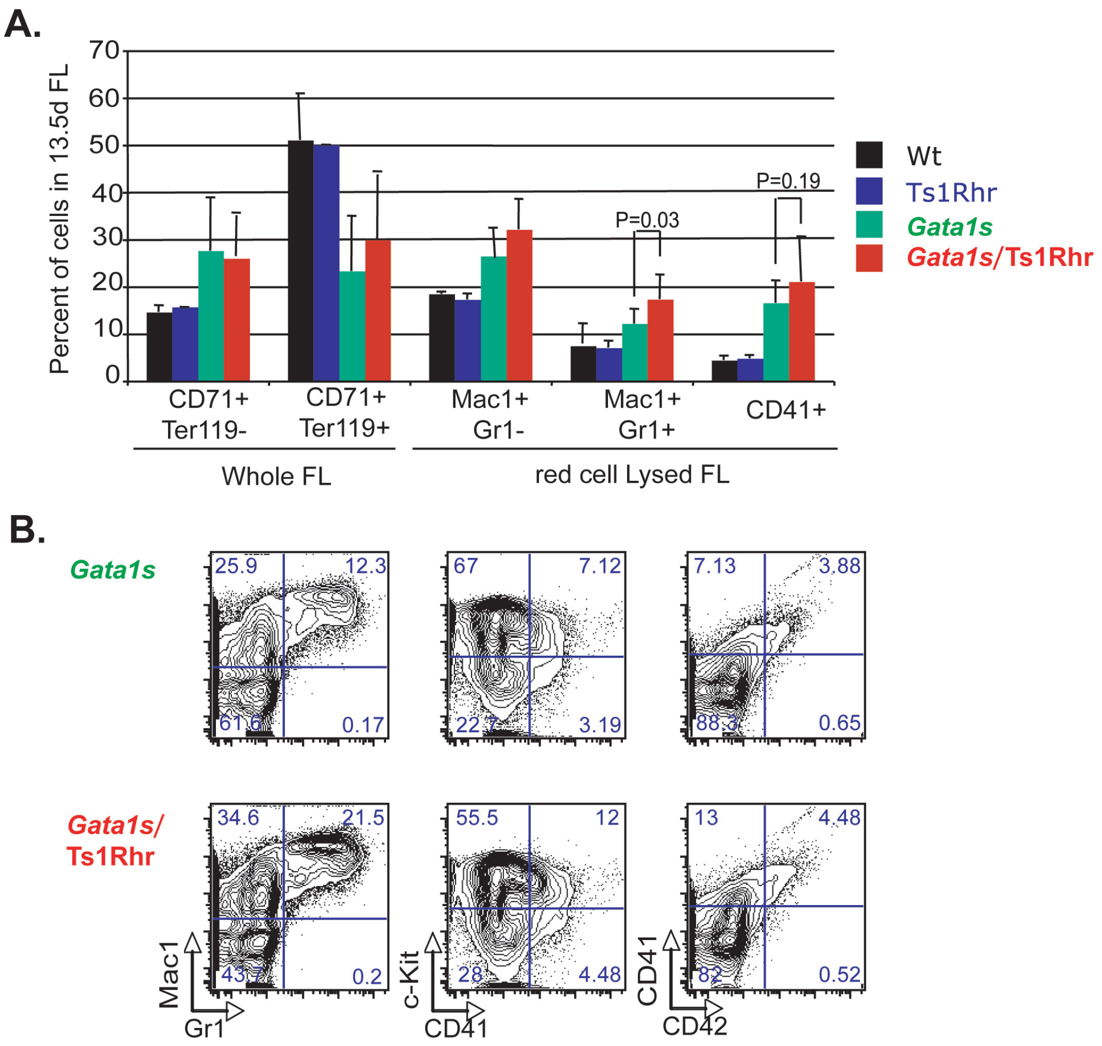
		Catalog number (Openbiosystems)		
Gene name	Accession number	shRNA - A	shRNA - B	**comments**
STCH	NM_006948	RHS4430-98486886		
SAMSN1	NM_022136	RHS4430-99147552	RHS4430-98521448	
GABPA	NM_002040	RHS4430-98512360	RHS4430-98894046	
BACH1	NM_032043	RHS4430-99139133	RHS4430-98818837	
SOD1	NM_000454	RHS4430-98850942		
SYNJ1	NM_203446	RHS4430-98911280	RHS4430-98902279	
C21ORF66	NM_013329	RHS4430-98817676	RHS4430-98818497	
IL10Rb	NM_000628	RHS4430-98853734	RHS4430-98850802	
IFNAR1	NM_000629	RHS4430-98852855	RHS4430-98514077	
IFNGR2	NM_005534	RHS4430-98894329	RHS4430-98842855	
DSCR1	NM_203418	RHS4430-99141160	RHS4430-99298620	
SETD4	NM_001007259	RHS4430-98894691		
CBR3	NM_001236	RHS4430-99159210		
DOPEY2	NM_005128	RMM4431-99010756		
MORC3	NM_015358	RHS4430-99161343		
CHAF1B	NM_005441	RHS4430-98851275		
HLCS	NM_000411	RHS4430-98913475	RHS4430-98704906	
DSCR5	NM_153681	RHS4430-98513697	RHS4430-99292024	
TTC3	NM_001001894	RHS4430-98851817	RHS4430-99139841	
DSCR3	NM_006052	RHS4430-98513710	RHS4430-99159927	
DYRK1A	NM_001396	RHS4430-99328993		
ERG	NM_001136154	RHS4430-98895011	RHS4430-98514732	
ETS2	NM_005239	RHS1764-9402477		subconed from pSM2c
DSCR2	NM_203433	RHS4430-99293698	RHS4430-98851553	
BRWD1	NM_033656	RHS4430-99150238	RHS4430-99294338	
HMGN1	NM_004965	RHS4430-98818607	RHS4430-99290749	
WRB	BC012415	RHS4430-99166550		
LCA5L	NM_152505	RHS4430-98818662	RHS4430-98485355	
SH3BGR	NM_001001713	RHS4430-99137372	RHS4430-98524364	
BACE2	NM_012105	RHS4430-98841733		
MX1	NM_002462	RHS4430-98893214	RHS4430-99138905	
FAM3B	NM_058186	RHS4430-99166795		

Supplemental figure 1

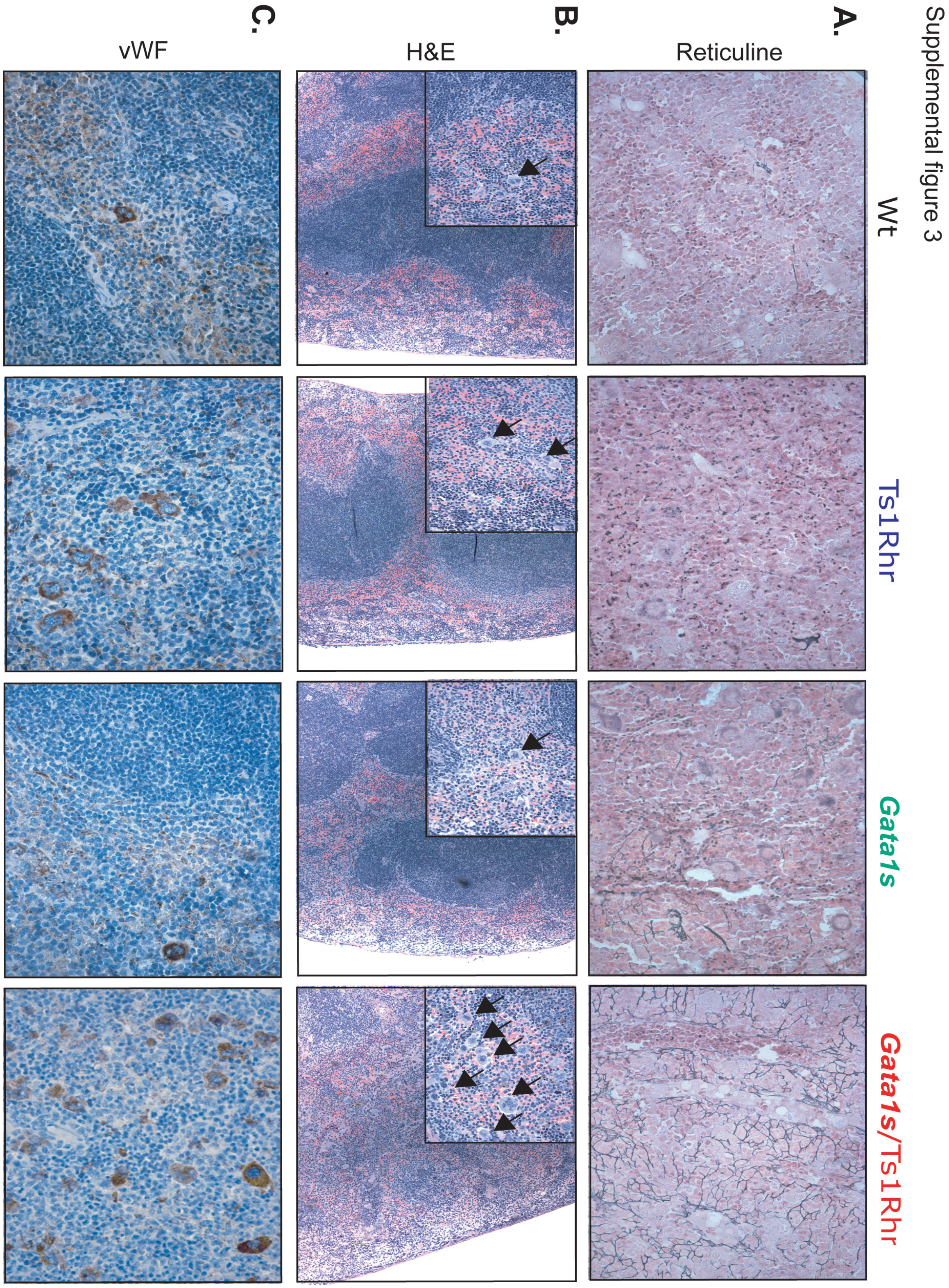


Supplemental figure 1: Hematopoietic disorders in the Ts1Rhr mice. (A) Monthly values of red blood cells (RBC) in the peripheral blood of Ts1Rhr mice and their euploid littermates. (B) Representative FACS plots showing increased percentage of CD41+ cells in bone marrow and spleen of old Ts1Rhr mice (>12 months old). Percentages of live cells are indicated. (C) Representative FACS plots for myeloid progenitors of 12-14 weeks old euploid and Ts1Rhr mice: CMP (Lin- c-kit+ Sca- FcγRII/III- CD34+), GMP (Lin- c-kit+ Sca- FcγRII/III+ CD34+), MEP (Lin- c-kit+ Sca- FcγRII/III- CD34-). Percentages of live cells are indicated. (D) CFU-assay colony number from wild-type and Ts1Rhr BM and SP cells. Data are shown as means +/- SD. (E) CFU-assay colony number from wild-type and Ts1Rhr fetal liver cells (13.5-14.5 days). Data are shown as means +/- SD. (F) Histograms representing percentages Thy1^{lo} Sca-1+ Lin- Mac-1+ CD4- (Long term reconstituting HSC as described in (33)) cell population in 13.5-14.5 days fetal livers. (Mean +/- SD). *p* value is shown.

Supplemental figure 2

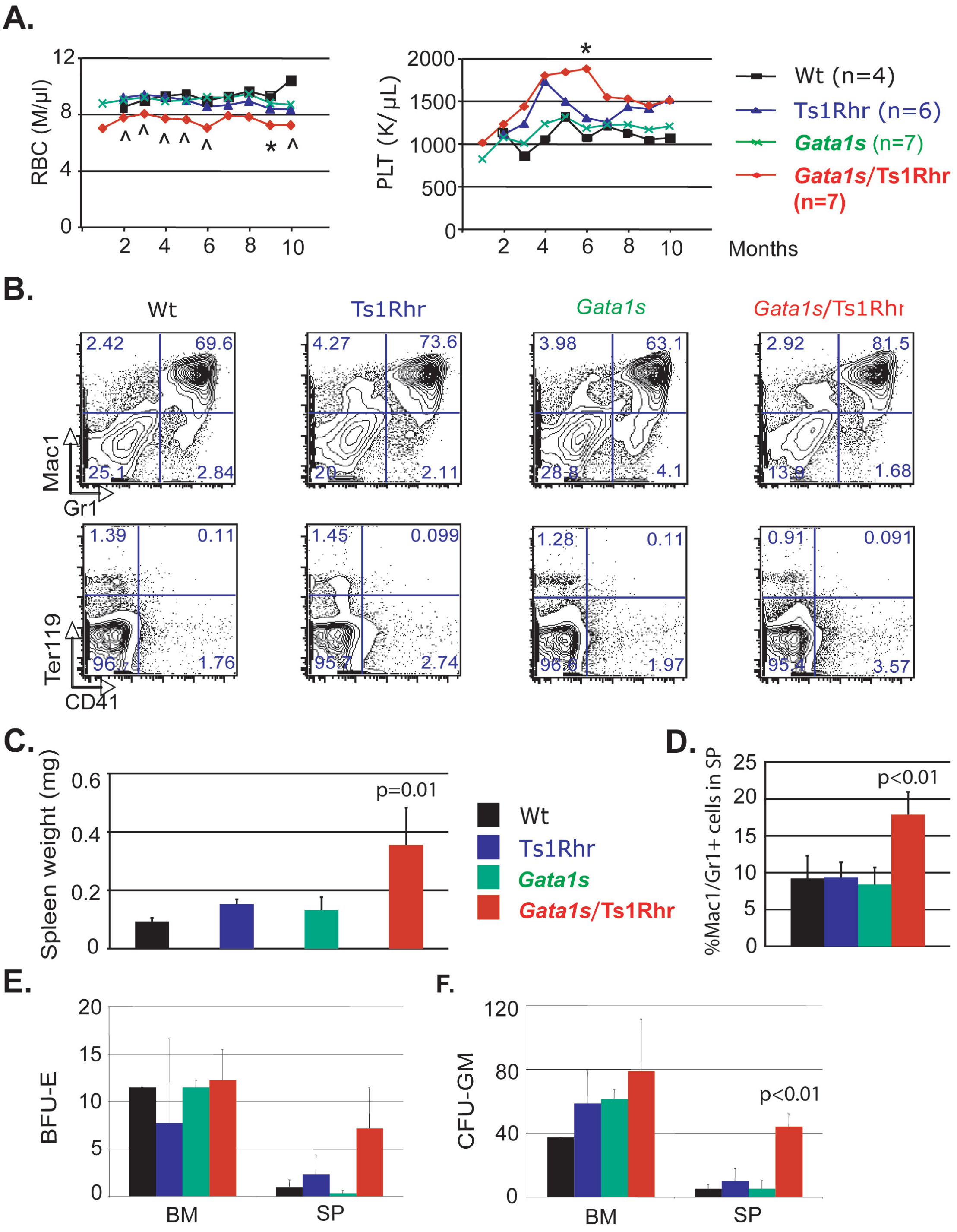


Supplemental figure 2: Fetal liver hematopoiesis is not significantly altered in doubly transgenic mice. (A) Histogram plots showing the proportion of myeloid cells in E13.5 Wt, Ts1Rhr, *Gata1s* and *Gata1s*/Ts1Rhr Fetal livers (FL) (n=2-10 per group). (B) Representative flow cytometry plots showing percentages of myeloid cells of E13.5 FL cells from *Gata1s* and *Gata1s*-Ts1Rhr mice. Percentages of live cells are indicated.



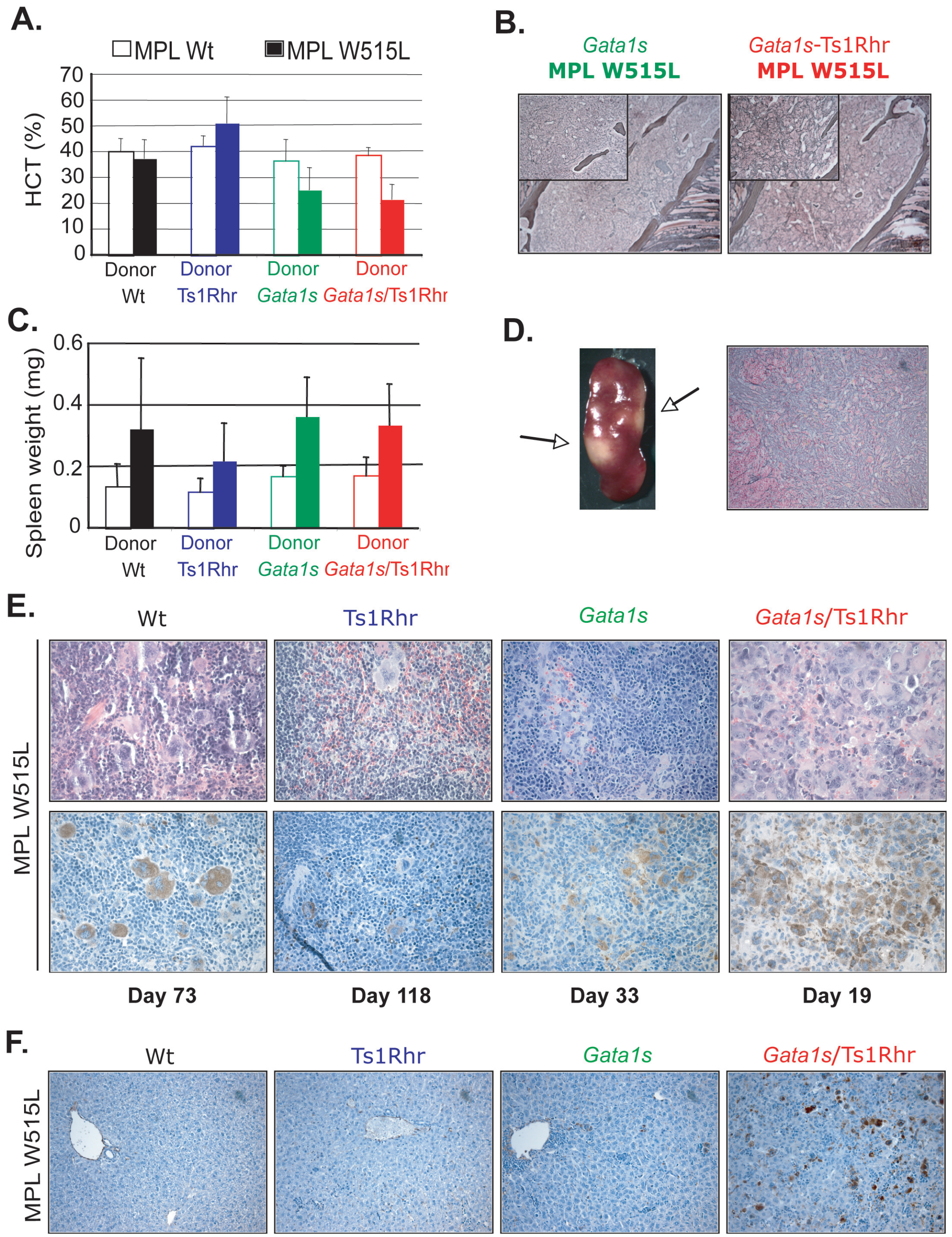
Supplemental figure 3: Higher resolution for Wt, Ts1Rhr, *Gata1s* and *Gata1s*/Ts1Rhr histological sections. (A-C) Bone marrow stained for reticulin in (A) (magnification 400X), Hematoxylin-eosin (H&E, in B) (100X, 400X) and von Willebrand Factor (vWF, in C) immunostaining (400X) from 6 month old mice.

Supplemental figure 4



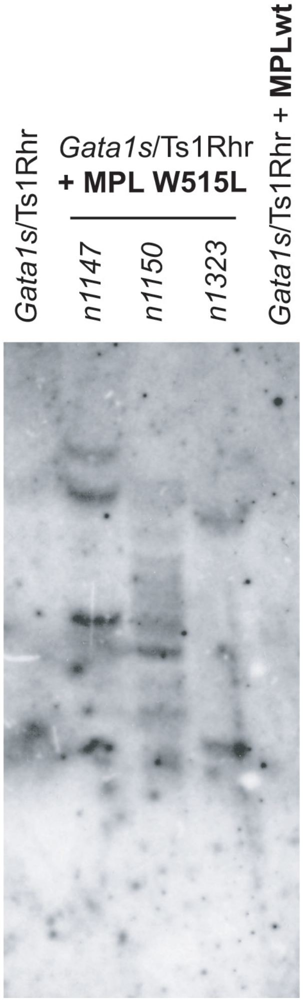
Supplemental figure 4: Altered hematopoiesis in adult *Gata1s*/Ts1Rhr doubly transgenic mice. (A) Monthly RBC and PLT values of Wt, Ts1Rhr, *Gata1s* and *Gata1s*/Ts1Rhr mice. n=number of mice. *$p<0.05$, ^$p<0.01$. (B) Representative flow cytometry plots of Mac1/Gr1 (top panel) and Ter119/CD41 (lower panel) BM cell populations of 6-month old mice. Percentages of live cells are indicated. (C) Histogram representing the average spleen weights of 6-month old mice (n=3-4 per group). (D) Proportion of the Mac1/Gr1 double positive cells in 6 months old spleens from each genotype (n=2-4 per group). (E) BFU-E and (F) CFU-GM colony forming assays from BM and SP cells from the 4 different genetic backgrounds (n=2-5 per group).

Supplemental figure 5



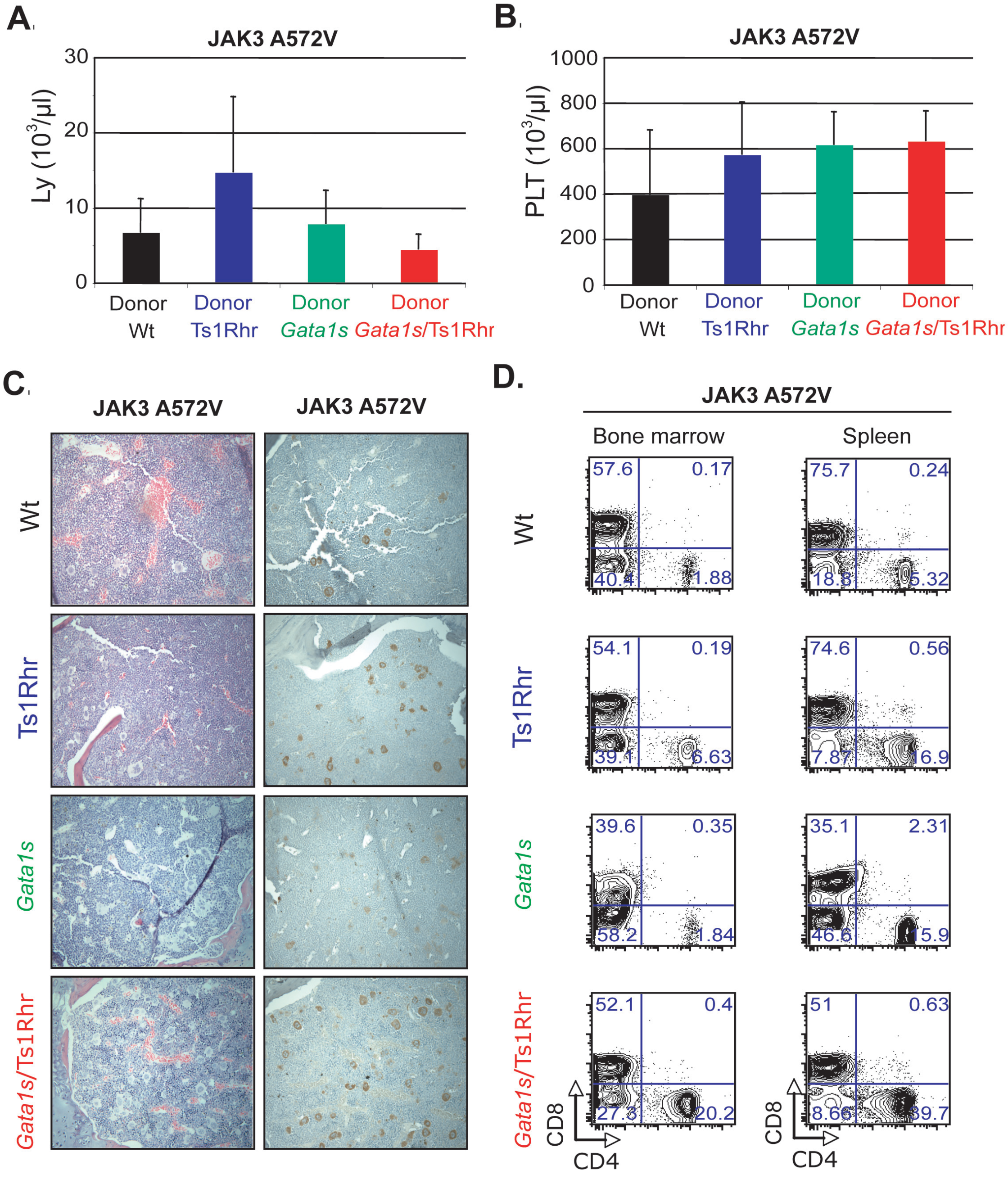
Supplemental figure 5: Ts1Rhr is required for development of fulminant megakaryoblastic leukemia with strong myelofibrosis by *Gata1s*/MPL W515L cells. (A) Hematocrit (HCT) value in the lethally irradiated recipient mice 4 weeks after transplantation of MPL wt or MPL W515L overexpressing cells from Wt, Ts1Rhr, *Gata1s* and *Gata1s*/Ts1Rhr donors (n=4-12 per group). (B) Reticulin stains of sternum section at 28 days (Magnification 100X-200X). (C) Spleen weight averages of recipient mice prior to sacrifice (n=2-12 per group). (D) Representative spleen and tumor (arrows) of the *Gata1s*/Ts1Rhr/MPL W515L-overexpressing recipient mice (left panel) and reticulin staining of the tumors (right panel, 100X). (E) H&E (upper panel) and vWF (lower panel) staining of spleen sections from moribund mice overexpressing MPL W515L (400X). (F) Representative liver sections of MPL W515L recipient mice 4 weeks post-BMT stained with vWF (200X).

Supplemental figure 6



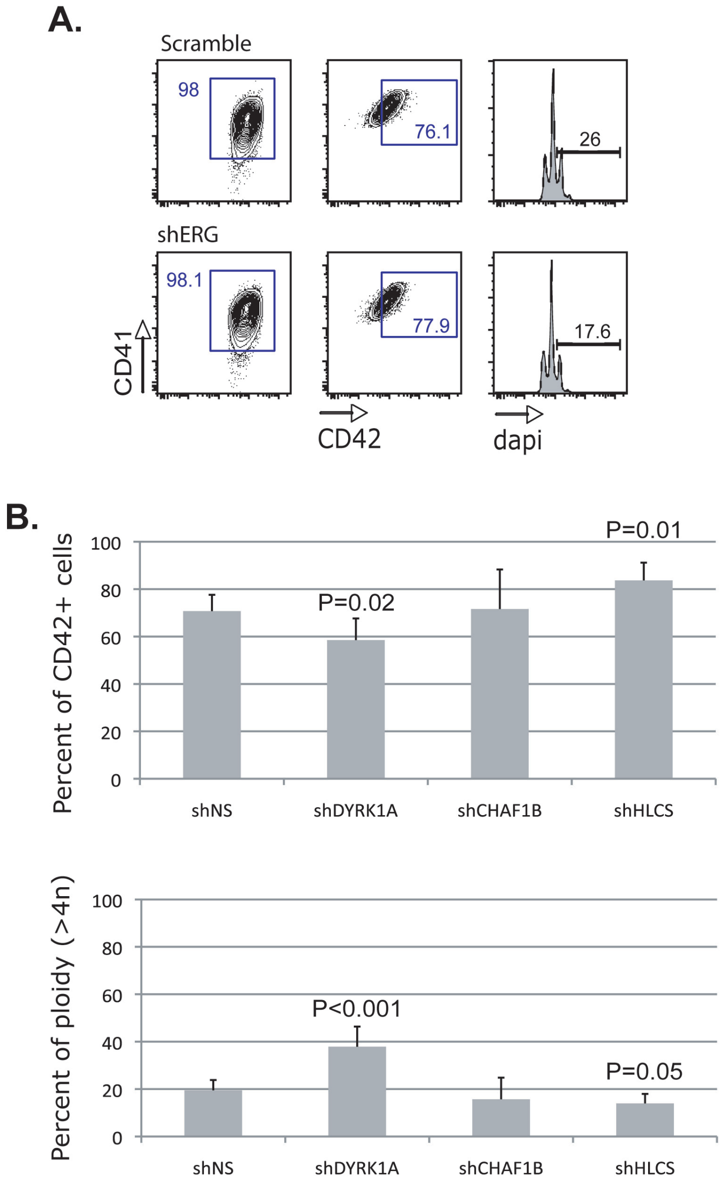
Supplemental figure 6: Clonality of the megakaryoblastic disorder observed in the moribund Gata1s/Ts1Rhr + MPL W515L mice. Southern blot analyses demonstrate an oligoclonal viral integration in the spleen of three representative triple mutant recipient mice 3-4 weeks after transplantation compared to Gata1s/Ts1Rhr and Gata1s/Ts1Rhr + MPL Wt.

Supplemental figure 7



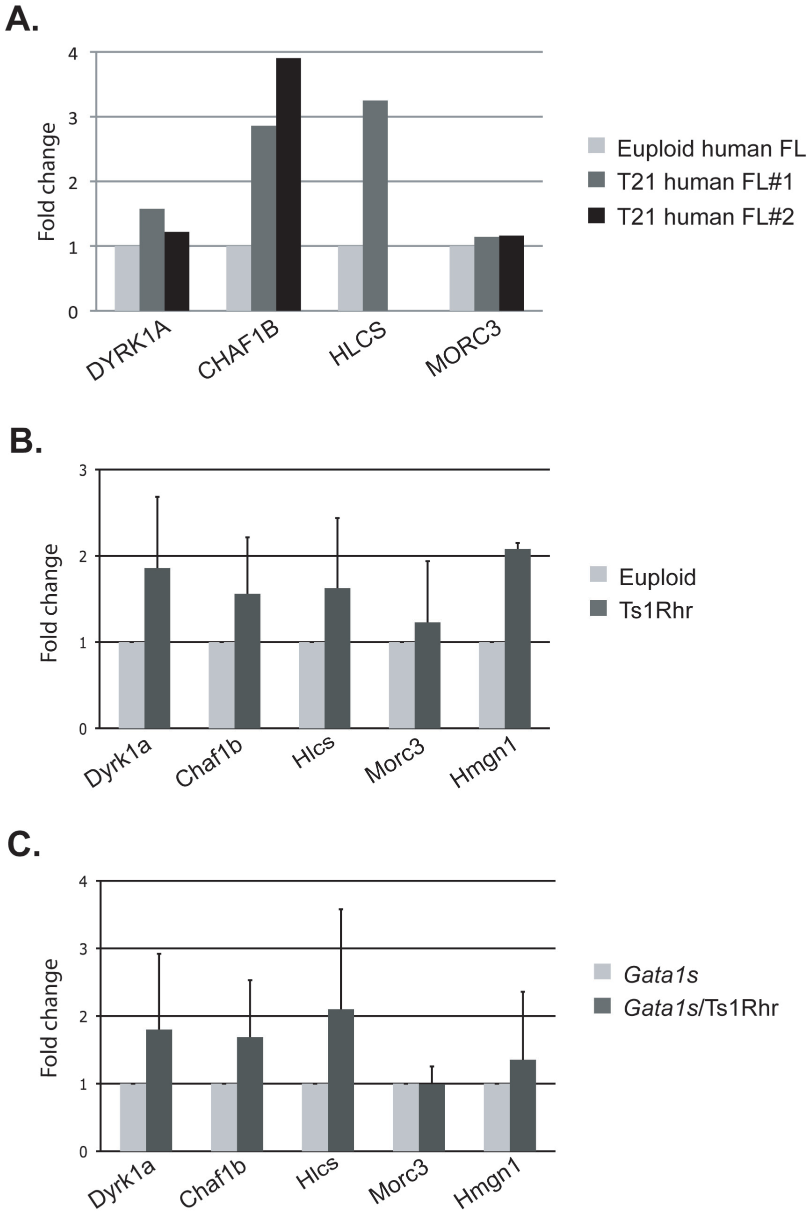
Supplemental figure 7: JAK3 A572V overexpression in Wt, Ts1Rhr, *Gata1s* or *Gata1s*/Ts1Rhr donor cells leads to a bi-phenotypic hematopoietic disorder in vivo. (A) Average lymphocyte (Ly) and (B) PLT counts of recipient mice 2 months after transplantation (n=3-4 per group). (C) Representative Hematoxylin-Eosin (Magnification 200X) and von Willebrand Factor (vWF) immunostaining (200X) of sternum sections from recipient mice 3 months post-transplantation. (D) Representative flow cytometry plots depicting the T cell lymphoproliferation observed in the BM and SP of JAK3 A572V overexpressing recipient mice 12 weeks after transplantation. Percentages of live cells are indicated.

Supplemental figure 8



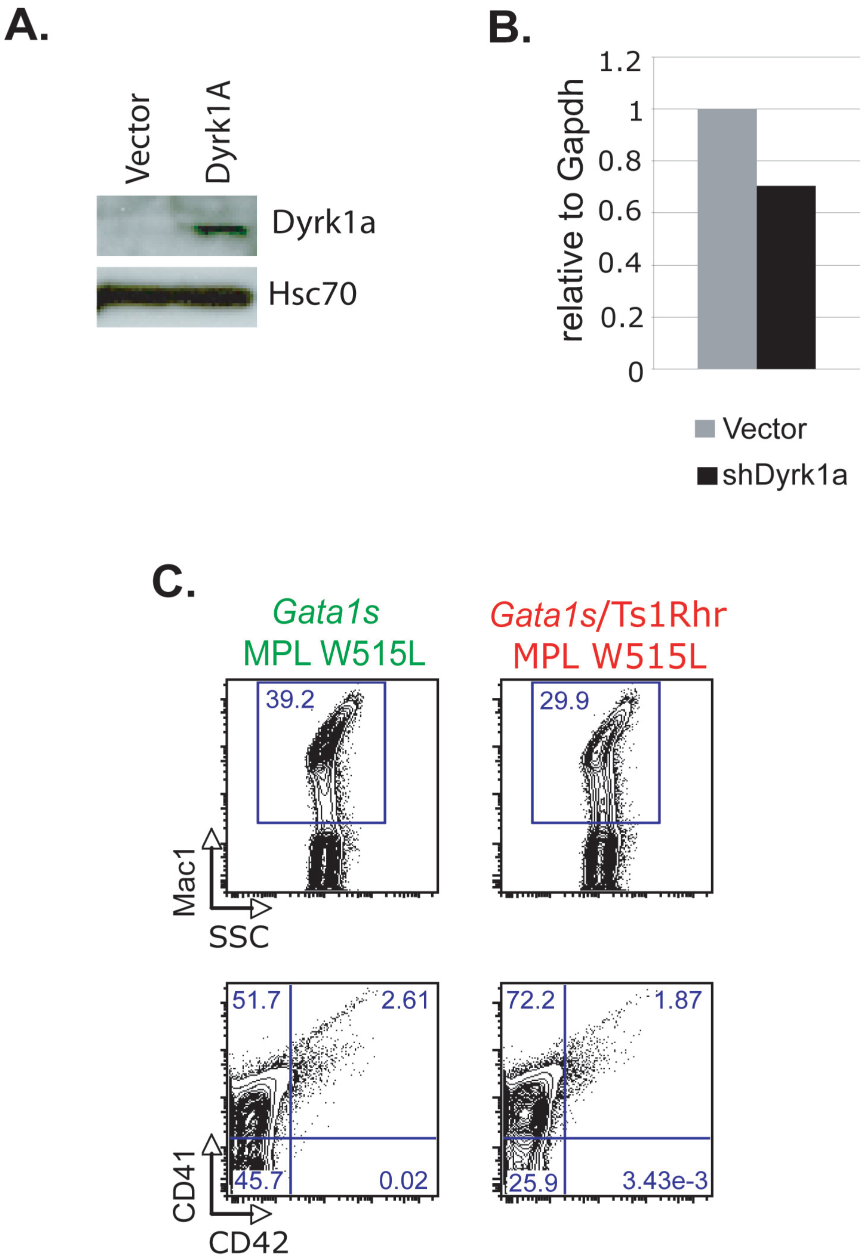
Supplemental figure 8: Knock-down of ERG, DYRK1A, CHAF1B and HLCS in the CMK cell lines. (A) FACS plots showing the effect of ERG Knock down in CMK cells during TPA-induced megakaryocytic differentiation (3 days). Percentages of live cells are indicated. (B) Histograms showing the percentages of CD42 expression and polyploidization (>4n) after TPA-induced differentiation of CMK cell lines knocked-down for DYRK1A, CHAF1B or HLCS compared to scramble vector (shNS). Percent of live cells are indicated. Data are shown as means +/- SD.

Supplemental figure 9



Supplemental figure 9: *DYRK1A*, *CHAF1B* and *HLCS* expression in human trisomic fetal livers and in our murine model. (A) Fold change gene expression values assessed by real-time PCR in day 14 megakaryocytes differentiated from trisomic or euploid mononuclear fetal liver cells. (B) Fold change expression values in >12 month old BM-megakaryocytes derived BM cells from Ts1Rhr mice (BSA gradient purified). (C) Fold change expression values in 6 months old *Gata1s*/Ts1Rhr BM derived megakaryocytes and BSA gradient purified compared to Euploid *Gata1s*.

Supplemental figure 10



Supplemental figure 10: Dyrk1a overexpression or knock-down validations, and phenotype of double and triple mutant cells. (A) Western blot showing DYRK1A overexpression in GFP-sorted infected bone marrow cells used in figure 4A-C. (B) Representative real-time analysis of DYRK1A expression in GFP-sorted Ts1Rhr bone marrow cells. (C) Mac+ and CD41+/CD42+ phenotypes of *Gata1s*/MPL W515L and *Gata1s*/Ts1Rhr/MPL W515L cell lines. Percentages of live cells are indicated.