Supplemental Tables

Table S1. DAVID: Gene sets significantly enriched in leukemic and non-leukemic LTHSC

Upregulated in Leukemic LTHSC		
Term	**Fold Enrichment**	**FDR p-value (Benjamini)**
GO:0007267~cell-cell signaling	5.279456	0.406176
Upregulated in Non-Leukemic LTHSC		
Term	**Fold Enrichment**	**FDR p-value (Benjamini)**
GO:0006955~immune response	5.896198	1.25E-10
mmu05322:Systemic lupus erythematosus	10.12886	3.22E-05
GO:0002449~lymphocyte mediated immunity	11.74529	4.30E-04
GO:0002504~antigen processing and presentation of peptide or polysaccharide antigen via MHC class II	31.32078	3.19E-04
GO:0002460~adaptive immune response based on somatic recombination of immune receptors built from immunoglobulin superfamily domains	10.62669	4.65E-04
GO:0002250~adaptive immune response	10.62669	4.65E-04
GO:0006952~defense response	3.98501	4.68E-04
GO:0048584~positive regulation of response to stimulus	6.39887	4.27E-04
GO:0002443~leukocyte mediated immunity	10.02969	4.13E-04
GO:0016064~immunoglobulin mediated immune response	12.5946	3.69E-04
GO:0019724~B cell mediated immunity	12.20707	4.06E-04
GO:0009617~response to bacterium	6.949091	3.84E-04
GO:0002684~positive regulation of immune system process	5.77762	6.22E-04
mmu04514:Cell adhesion molecules (CAMs)	6.774498	4.71E-04
GO:0002495~antigen processing and presentation of peptide antigen via MHC class II	30.99453	0.001308318
GO:0019886~antigen processing and presentation of exogenous peptide antigen via MHC class II	30.99453	0.001308318
GO:0002252~immune effector process	7.084463	0.002894512
GO:0050778~positive regulation of immune response	6.563547	0.004630398
GO:0002478~antigen processing and presentation of exogenous peptide antigen	21.56141	0.004814384
GO:0019884~antigen processing and presentation of exogenous antigen	17.71116	0.010013394
mmu04612:Antigen processing and presentation	8.025175	0.005460354
mmu05332:Graft-versus-host disease	10.79248	0.004164554
GO:0051085~chaperone mediated protein folding requiring cofactor	33.06083	0.011658489
GO:0002253~activation of immune response	8.072993	0.011968716
mmu05310:Asthma	15.80716	0.004067517
mmu04940:Type I diabetes mellitus	9.935931	0.004110199
GO:0051084~'de novo' posttranslational protein folding	28.33785	0.016963038
GO:0006458~'de novo' protein folding	28.33785	0.016963038
GO:0048002~antigen processing and presentation of peptide antigen	14.16893	0.019315376
GO:0007155~cell adhesion	2.828734	0.022835885
GO:0022610~biological adhesion	2.823701	0.022213179

GO:0045087~innate immune response	6.488574	0.030072925
GO:0042742~defense response to bacterium	6.428494	0.030265528
mmu04640:Hematopoietic cell lineage	7.451948	0.013270462
mmu04672:Intestinal immune network for IgA production	9.659933	0.016878853

Table S2. GSEA analysis: Gene sets significantly enriched in non-leukemic LTHSC

NAME	NES	FDR q-val
YU_CMYC_DN	-2.22178	0.002627
HSC_MATURE_ADULT	-2.11287	0.007185
UVB_NHEK1_C6	-2.06658	0.00916
UVB_NHEK1_DN	-2.04175	0.011458
ICHIBA_GVHD	-2.0221	0.012797
HSC_MATURE_SHARED	-1.97993	0.018213
SANA_IFNG_ENDOTHELIAL_UP	-1.95168	0.025419
UVC_TTD_4HR_DN	-1.91199	0.039235
LAL_KO_3MO_UP	-1.88179	0.052497
LAL_KO_6MO_UP	-1.86915	0.057297
ADIP_VS_FIBRO_DN	-1.85287	0.063143
UVC_TTD_ALL_DN	-1.85282	0.057989
BENNETT_SLE_UP	-1.84236	0.062045
HSC_MATURE_FETAL	-1.83538	0.06338
BYSTROM_IL5_DN	-1.82092	0.070787
WANG_MLL_CBP_VS_GMP_UP	-1.79554	0.089286
ET743_SARCOMA_24HRS_DN	-1.78936	0.089892
KIM_TH_CELLS_UP	-1.76246	0.11554
TAKEDA_NUP8_HOXA9_16D_DN	-1.75964	0.11351
WANG_HOXA9_VS_MEIS1_UP	-1.74964	0.121449
HBX_HCC_DN	-1.74959	0.115726
CARIES_PULP_HIGH_UP	-1.72723	0.142555
CPR_LOW_LIVER_DN	-1.72618	0.137712
HSC_LATEPROGENITORS_SHARED	-1.72253	0.137333
BCRABL_HL60_CDNA_DN	-1.72078	0.134224
AMIPATHWAY	-1.71396	0.138888
LIAN_MYELOID_DIFF_GRANULE	-1.71387	0.133987
CSKPATHWAY	-1.71174	0.132035
GLYCOGEN_METABOLISM	-1.70573	0.136769
PAR1PATHWAY	-1.70408	0.134369
CREB_BRAIN_8WKS_DN	-1.70391	0.13033
HSC_LATEPROGENITORS_FETAL	-1.69944	0.131913
HSC_LATEPROGENITORS_ADULT	-1.69181	0.13808
STANELLE_E2F1_UP	-1.68417	0.144567
EGFPATHWAY	-1.68242	0.142979
NO2IL12PATHWAY	-1.68125	0.14056
UVC_XPCS_ALL_DN	-1.67672	0.142599
WIELAND_HEPATITIS_B_INDUCED	-1.67125	0.146749
IL6PATHWAY	-1.67018	0.144048
ET743_SARCOMA_48HRS_DN	-1.6677	0.143596
UVC_XPCS_4HR_DN	-1.66412	0.145515
CHANG_SERUM_RESPONSE_DN	-1.65985	0.148077
IL2PATHWAY	-1.65683	0.149137
HDACI_COLON_SUL48HRS_UP	-1.65207	0.151895

UVB_NHEK3_C6	-1.64995	0.152026
FLECHNER_KIDNEY_TRANSPLANT_REJECTION_UP	-1.64715	0.152449
HDACI_COLON_SUL_UP	-1.64388	0.15394
KERATINOCYTEPATHWAY	-1.63434	0.163637
BRACX_UP	-1.62667	0.172436
ET743_SARCOMA_DN	-1.62235	0.175756
METPATHWAY	-1.61809	0.179679
GREENBAUM_E2A_DN	-1.61467	0.181793
UVC_XPCS_8HR_DN	-1.61288	0.181621
PDGFPATHWAY	-1.60886	0.185289
RADAEVA_IFNA_UP	-1.60831	0.182723
TRANSLATION_FACTORS	-1.60587	0.183301
NKCELLSPATHWAY	-1.60368	0.18375
KNUDSEN_PMNS_DN	-1.59719	0.191888
IDX_TSA_DN_CLUSTER2	-1.59395	0.194283
RHOPATHWAY	-1.59347	0.191696
IDX_TSA_DN_CLUSTER5	-1.59278	0.18954
UV-4NQO_FIBRO_UP	-1.59257	0.186671
XU_CBP_DN	-1.57263	0.216779
IFNA_HCMV_6HRS_UP	-1.56577	0.225417
ZHAN_MM_CD138_LB_VS_REST	-1.56304	0.226705
PYK2PATHWAY	-1.56252	0.224195
DAVIES_MGUS_MM	-1.56122	0.223292
IGF1PATHWAY	-1.56084	0.220641
TSA_CD4_UP	-1.55814	0.221899
IFNALPHA_HCC_UP	-1.54578	0.241903
COCAINE_BRAIN_4WKS_UP	-1.54515	0.239505
TSADAC_PANC50_UP	-1.54063	0.245221
EDG1PATHWAY	-1.53988	0.24309
TALL1PATHWAY	-1.5379	0.243432
CMV_HCMV_TIMECOURSE_18HRS_UP	-1.53704	0.242093

Gene sets with FDR<0.25 are shown. None of the gene sets enriched in leukemic LTHSC reached this level of significance.

Table S3: TaqMan® Gene Expression Assay used for Q-PCR analysis

Gene name	**Assay ID**
BMI1	Mm03053308_g1*
NOXA/ Pmaip1	Mm00451763_m1*
P16INK4A-Cdkn2a	Mm00494449_m1
P21 - Cdkn1a	Mm00432448_m1*
GLI1	Mm00494645_m1
GLI2	Mm01293111_m1
Trp53	Mm01731290_g1
CCND1	Mm00432358_g1
HES1	Mm01342805_m1
PPARD	Mm01305434_m1
PUMA / BCL2 binding component 3	Mm00519268_m1*
Integrin alpha 4	Mm00439770_m1*
CD47	Mm00495005_m1*
SIRT1	Mm01168521_m1
PTCH1	Mm01306905_m1
PTEN	Mm00477208_m1*
MDM2	Mm01233136_m1*
FOXO1	Mm00490672_m1*
BAX / BCL2-associated X protein	Mm00432050_m1*
CXCR4	Mm01292123_m1
HPRT1	Mm00446968_m1*
FZD7	Mm00433409_s1
NECDIN	Mm02524479_s1*
GFI-1	Mm00515855_m1*
DR5/Tnfrsf10b	Mm00457866_m1*
CDH2 (N-Cadherin)	Mm00483213_m1
MPL	Hs00180489_m1
Bcl2l1(Bcl-xl)	Mm00437783_m1
Cdkn1a	Mm00432448_m1*
Cdkn1b	Mm00438168_m1
Cdkn1c	Mm01272135_g1
Cdkn2c	Mm00483243_m1
Cdkn2d	Mm00486943_m1
Gata2	Mm00492301_m1
HIF2a(Epas1)	Mm01236112_m1
Hoxb4	Mm00657964_m1
Hoxa9	Mm00439364_m1
Itga2b(CD41)	Mm00439741_m1
c-Myc	Mm00487804_m1
Mpl	Mm00440310_m1
Osm	Mm01193966_m1

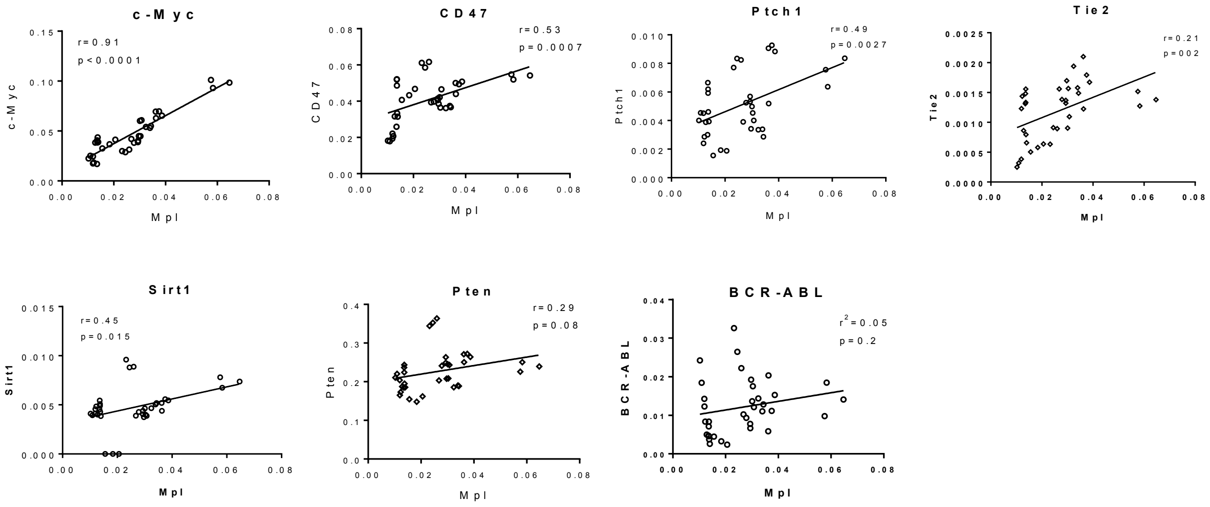
Pim1	Mm00435712_m1
Slamf1	Mm00443316_m1
Tek(Tie2)	Mm00443242_m1
Vwf	Mm00550376_m1
B2M	Mm00437762_m1

Table S4. Antibodies used.

Target	**Targeted species**	**Conjugate**	**Source**	**Catalog number**	**Clone**
CD3	mouse	Biotin	eBioscience	13-0031-85	17A2
CD4	mouse	Biotin	eBioscience	13-0041-85	GK1.5
CD8	mouse	Biotin	eBioscience	13-0083-85	53-6.7
B220	mouse	Biotin	eBioscience	13-0452-85	RA3-6B2
CD19	mouse	Biotin	eBioscience	13-0193-85	eBio1D3 (1D3)
IgM	mouse	Biotin	eBioscience	13-5790-85	eB121-15F9
Gr-1	mouse	Biotin	eBioscience	13-5931-85	RB6-8C5
CD11b	mouse	Biotin	eBioscience	13-0112-85	M1/70
NK1.1	mouse	Biotin	eBioscience	13-5941-85	PK136
Ter119	mouse	Biotin	eBioscience	13-5921-85	TER-119
Flt3	mouse	Biotin	eBioscience	13-1351-85	A2F10
Flt3	mouse	PE	eBioscience	12-1351-82	A2F10
Sca-1	mouse	PE-Cy7	eBioscience	25-5981-82	D7
CD117	mouse	APC-efluor780	eBioscience	47-1172-82	ACK2
CD150	mouse	PerCP-Cy5.5	Biolegend	115922	TC15-12F12.2
CD48	mouse	APC	eBioscience	17-0481-82	HM48-1
CD45.1	mouse	PE-Cy7	eBioscience	25-0453-82	A20
CD45.1	mouse	PerCP-Cy5.5	eBioscience	45-0453-80	A20
CD45.2	mouse	FITC	eBioscience	11-0454-85	104
CD45.2	mouse	eFluor® 450	eBioscience	48-0454-82	104
CD45	mouse	PE-Cy7	eBioscience	25-0451-82	30-F11
CD45	human	FITC	eBioscience	11-9459-42	2D1
CD45	human	PerCP-Cy5.5	eBioscience	45-9459-42	2D1
CD14	human	Biotin	ebioscience	13-0149-82	61D3
CD14	human	APC-efluor780	ebioscience	47-0149-42	61D3
CD33	human	PE	BD	347787	P67.6
CD3	human	Biotin	ebioscience	13-0037-80	OKT3
CD3	human	PerCP-Cy5.5	ebioscience	45-0037-42	OKT3
CD19	human	Biotin	ebioscience	13-0199-82	HIB19
CD19	human	PE-Cy7	ebioscience	25-0199-42	HIB19
CD34	human	FITC	ebioscience	11-0349-42	4H11
CD34	human	PE-Cy7	ebioscience	25-0349-42	4H11
CD38	human	PE	ebioscience	12-0388-42	HB7
CD38	human	FITC	ebioscience	11-0388-42	HB7
CD38	human	APC	ebioscience	17-0389-42	HIT2
CD90	human	PerCP-Cy5.5	ebioscience	45-0909-42	eBio5E10 (5E10)
CD90	human	PE	ebioscience	12-0909-42	eBio5E10 (5E10)
MPL	human	APC	BD	562199	1.6.1

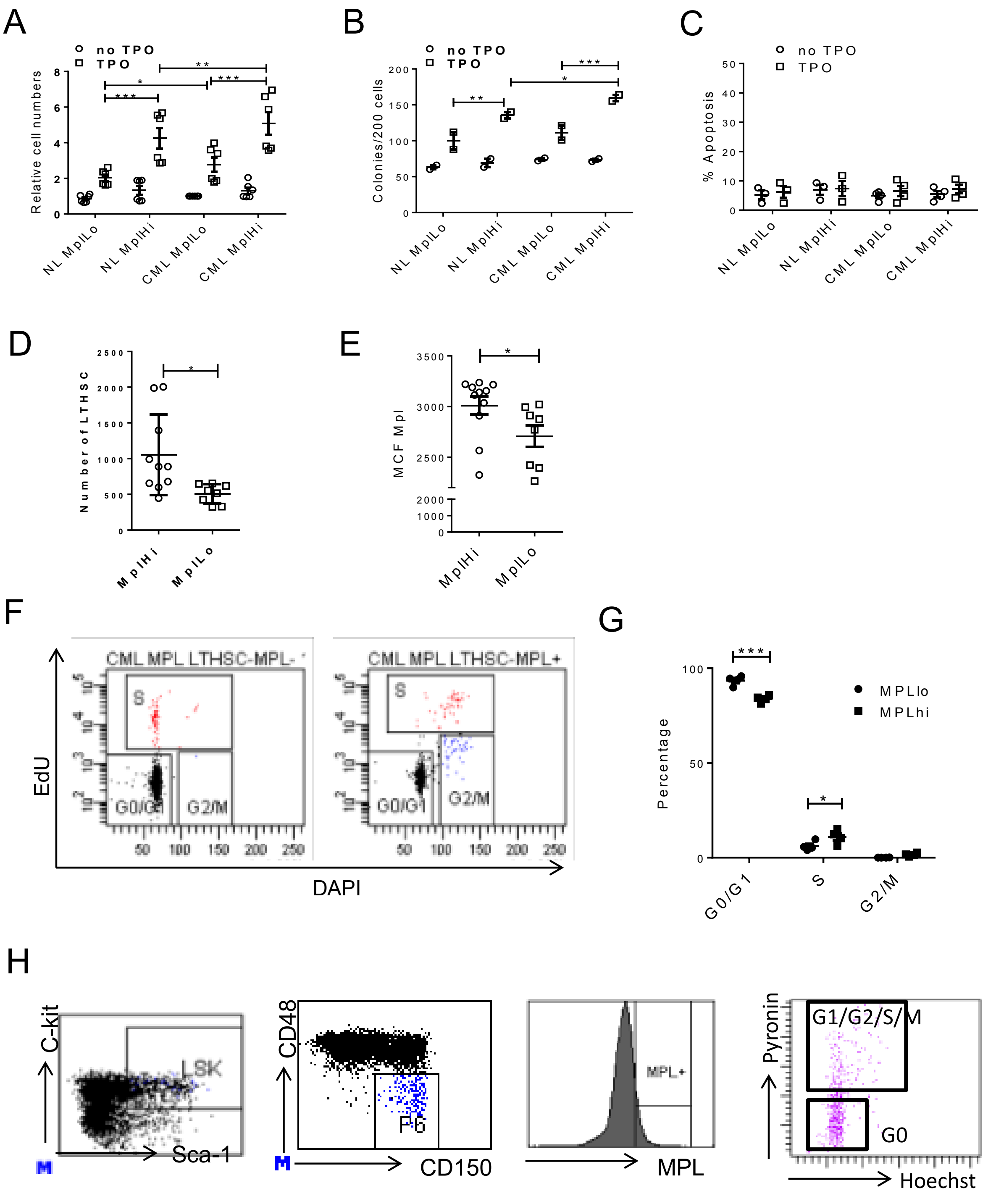
CD229	human	PE	Biolegend	326108	Hly-9.1.25
CD41	human	PE	BD	558040	MWReg30
Ki67	human	FITC	BD	556026	B56
Ki67	human	PE	BD	556027	B56
P-STAT3	human	PerCP-Cy5.5	BD	560114	4/P-STAT3
P-STAT5	human	Alex 488	BD	562075	47/Stat5(pY694)
P-STAT5	human	Alex 647	BD	562076	47/Stat5(pY694)
p-ERK	human	PE	Ebioscience	12-9109-42	MILAN8R
p-CRKL	human/mouse	Rabbit	cell signaling	3181	
Goat Anti-Rabbit IgG	rabbit	Alexa Fluor 488	Jackson ImmunoResearch	111-545-144	
Goat Anti-Rabbit IgG	rabbit	Alexa Fluor® 647	Jackson ImmunoResearch	111-605-144	

Supplemental Figure 1



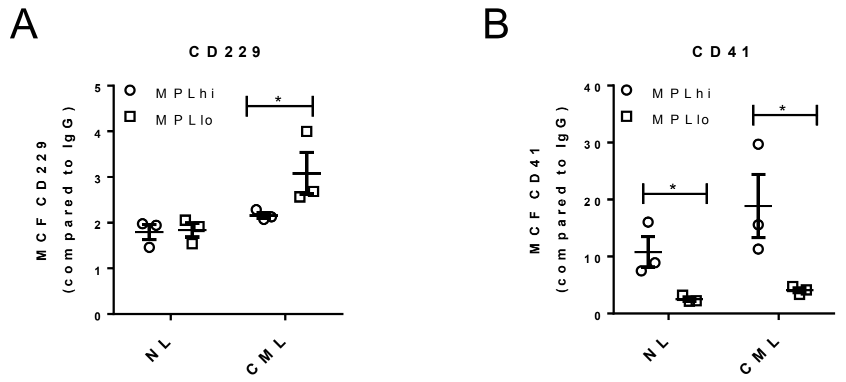
Supplemental Figure S1 (related to Figure 2). Gene expression patterns in LTHSC from leukemic and non-leukemic mice. Correlation between mRNA expression for MPL and c-Myc, CD47, Ptch1, Tie2, Sirt1, Pten, and BCR-ABL.

Supplemental Figure 2



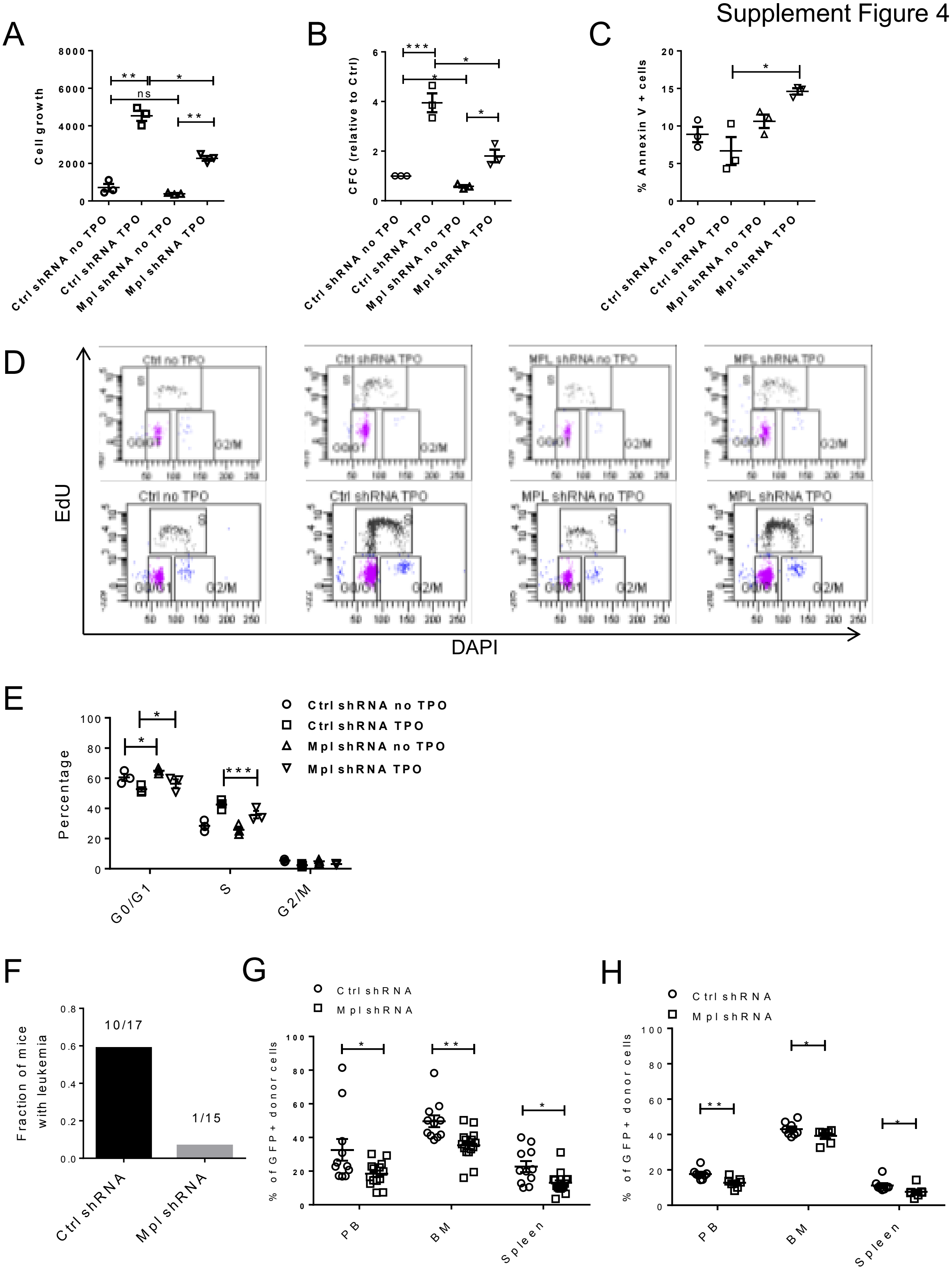
Supplemental Figure S2 (related to Figure 3). Enhanced leukemogenic capacity of MplHi LTHSC. Cell growth (A), CFC frequency (B) and apoptosis (C) of normal and CML MplHi and MplLo LTHSC after culture with and without THPO (10ng/ml) for 72 hours. Number of LTHSC (D), and Mpl expression on LTHSC (E), in BM of recipient mice transplanted with CML MplHi and MplLo LTHSC. Flow cytometry plots (F) and summary graphs (G) showing cell cycle status of CML MplHi and MplLo LTHSC determined by EdU and DAPI labeling. Flow cytometry selection of Hoechst^{lo} Pyronin^{lo} (quiescent) and Hoechst$^{hi/lo}$ Pyronin^{hi} (proliferating) CD45.1 MPLhi LTHSC (H).

Supplemental Figure 3



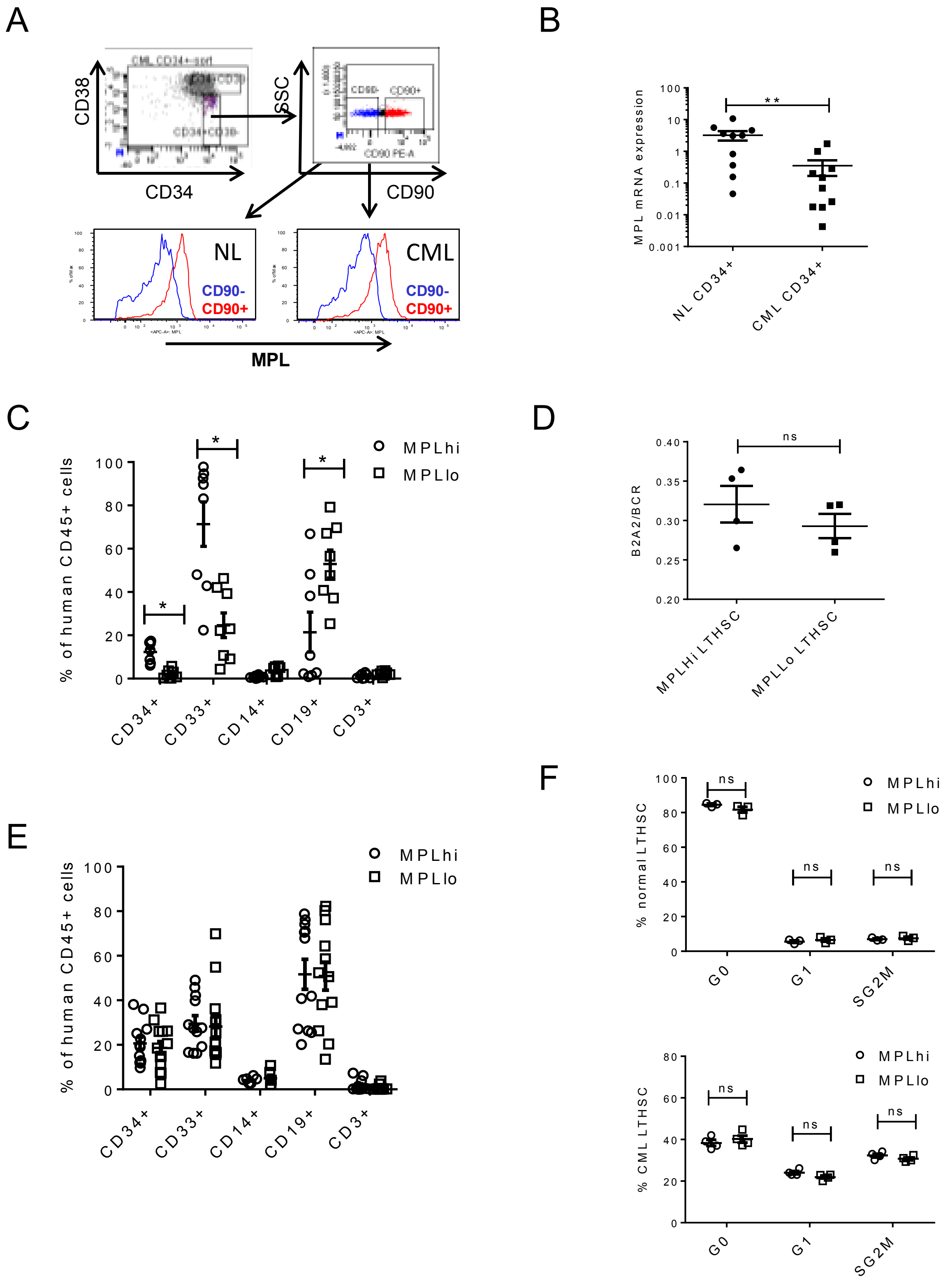
Supplemental Figure S3. (Related to Figure 4) Expression of megakaryocytic-lineage, stem cell and cell cycle regulatory genes in MplHi LTHSC. Combined results for cell surface expression of CD229 (A) and CD41 (B) on normal and CML MplHi and MplLo LTHSC cells.

Supplement Figure 4



Supplemental Figure S4. (Related to Figure 5) RNAi mediated knockdown of Mpl expression reduces leukemogenic capacity of CML LTHSC. Effect of Mpl shRNA expression on cell growth (A), CFC (B), apoptosis (C), and cell cycling measured by EDU staining (D, E) are shown. The fraction of recipient mice developing CML within 16 weeks after transplantation with Ctrl and Mpl- shRNA expressing cells is shown in (F).Engraftment of GFP+ CML (G) and normal (H) donor cells in the PB, BM and spleens of recipient mice at 16 weeks.

Supplemental Figure 5



Supplemental Figure S5. (Related to Figure 7) Human CML MPL^{Hi} LTHSC show enhanced proliferative and regenerative capacity. (A) Flow cytometry plot showing MPL expression in human normal and CML CD34+CD38-CD90+ LTHSC cells. (B) MPL mRNA expression in human normal and CML CD34+ cells analyzed by Q-RT-PCR. (C)

Human CD45+ cell subpopulations engrafted in BM of NSG mice transplanted with CML LTHSC at 16 weeks. (D) BCR-ABL mRNA expression in human cells engrafted in the BM of NSG mice transplanted with CML LTHSC at 16 weeks analyzed by RT-Q-PCR. (E) Human CD45+ cell subpopulations engrafted in BM of NSG mice transplanted with normal LTHSC at 16 weeks. (F) Cell cycling of normal and CML Mpl$_{Hi}$ and Mpl$_{Lo}$ LTHSC cultured in SFEM medium for 72 hours without THPO