SUPPLEMENTARY FIGURES

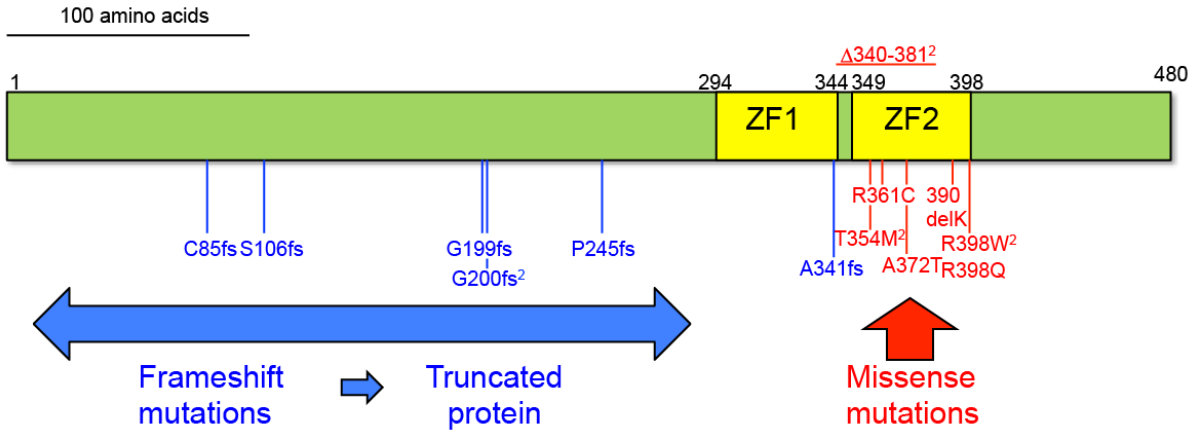
Supplementary Table 1. Antibodies used for flow cytometry.

Protein	Fluorochrome	Clone	Isotype	Supplier
CCR7	PE-Cy7	G043H7	Ms IgG2a, κ	Biolegend
CD1c	PeCy7	L161	Ms IgG_1,κ	BioLegend
CD3	FITC	SK7	Ms IgG_1,κ	BD
CD3	PE	SK7	Ms IgG_1, κ	BD
CD3	V500	UCHT1	Ms IgG_1, κ	BD
CD4	FITC	SK3	Ms IgG_1, κ	BD
CD4	PE-Cy7	SK3	Ms IgG_1, κ	BD
CD4	PE	SK3	Ms IgG_1, κ	BD
CD8	APC	SK1	Ms IgG_1, κ	BD
CD8	APC-Cy7	SK1	Ms IgG_1, κ	BD
CD11c	V450	B-Ly6	Ms IgG_1,κ	BD
CD14	BV650	M5E2	Ms IgG_{2a}, κ	BioLegend
CD16	APC-H7	3G8	Ms IgG_1,κ	BD
CD19	FITC	4G7	Ms IgG_1,κ	BD
CD20	FITC	L27	Ms IgG_1,κ	BD
CD25	APC-Cy7	M-A251	Ms IgG_1,κ	BD
CD27	BV421	O323	Ms IgG_1,κ	Biolegend
CD34	PE	8G12	Ms IgG_1,κ	BD
CD38	PE-Cy7	HB7	Ms IgG_1,κ	BD
CD45RA	APC-Cy7	HI100	Ms IgG_{2b}, κ	Biolegend
CD45RA	Q605	MEM-56	Ms IgG_{2b}, κ	Invitrogen

CD56	FITC	NCAM16.2	Ms IgG_{2b}, κ	BD
CD56	BV421	HCD56	Ms IgG_1, κ	Biolegend
CD56	PERCPCy5.5	HCD56	Ms IgG_{2a}, κ	Biolegend
CD56	PERCPCy5.5	MEM-188	Ms IgG_{2a}, κ	Biolegend
CD62L	PE-Cy7	DREG-56	Ms IgG_1, κ	Biolegend
CD123	PERCPCy5.5	7G3	Ms IgG_{2a}, κ	BD
CD127	APC	A019D5	Ms IgG_1	Biolegend
CD141	APC	AD5-14H12	Ms IgG1	Miltenyi
CD158e1/e2	PE	Z27.3.7	Ms IgG_1	Beckman C
CD158a,h	PE	EB6.B	Ms IgG_1	Beckman C
CD158 b1/b2,j	PE	GL183	Ms IgG_1	Beckman C
CD161	BV421	HP-3G10	Ms IgG_1, κ	Biolegend
HLA-DR	V500	L243	Ms IgG_{2a}, κ	BD
HLA-DR	A700	G46-6	Ms IgG_{2b}, κ	BD
NKG2A	APC	Z199	Ms IgG_{2b}, κ	Beckman C
NKG2C	PerCP	134591	Ms IgG_1, κ	R&D
TCRVα24Jα18	PE	6B11	Ms IgG_1, κ	Biolegend
TCR$\gamma\delta$	FITC	B1	Ms IgG_1, κ	BD
TCRVα7.2	APC	3C10	Ms IgG_1, κ	Biolegend

Supplementary Figure 1. ***Distribution of GATA2 mutation***

Summary of distribution of *GATA2* mutations in the cohort. ZF1 and ZF2 indicate the two zinc finger regions. Superscript numerals indicate the more than one pedigree with the same mutation.



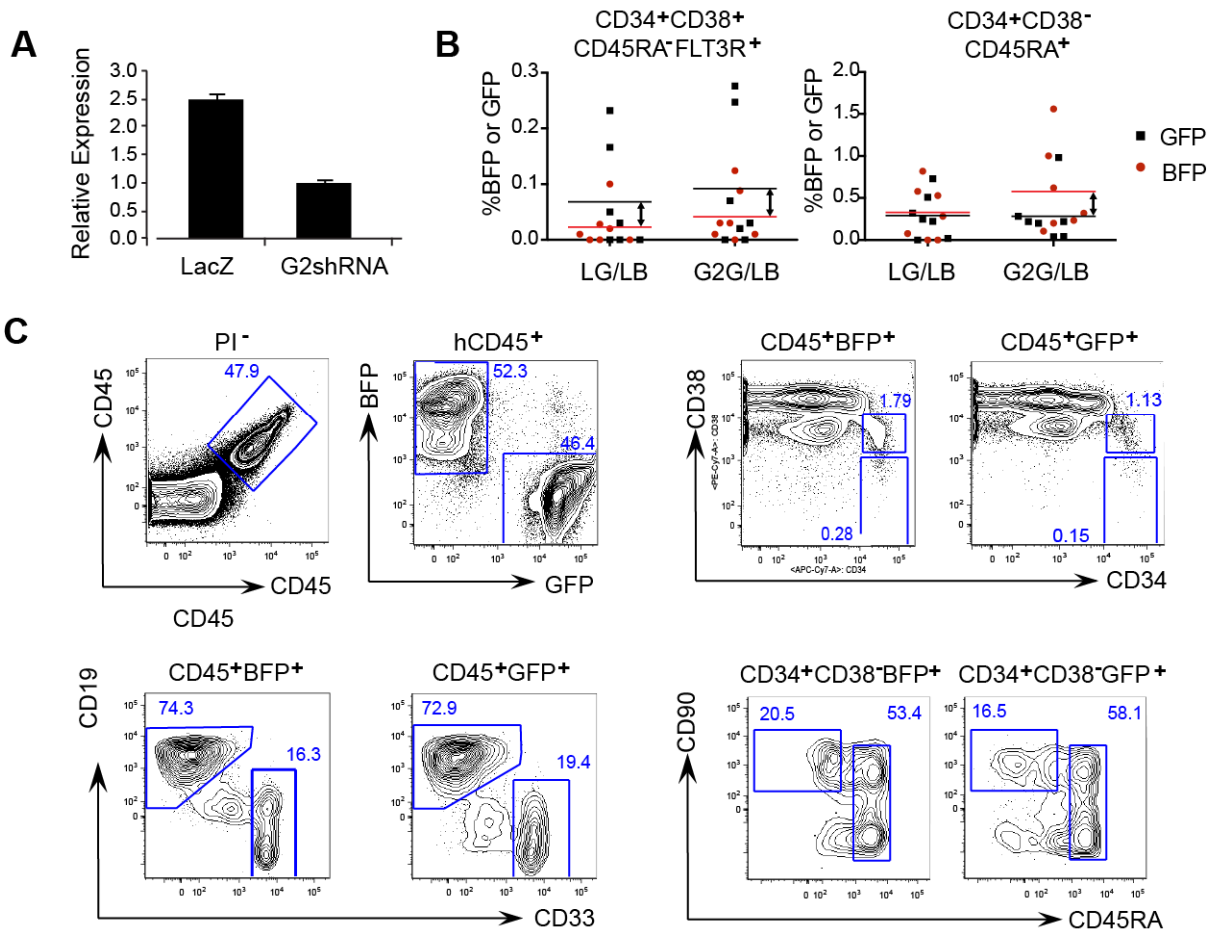
Supplementary Figure 2. Effect of *GATA2 knock-down on human progenitors.*

Short hairpin RNA (shRNA) lentiviral vectors were constructed to knock-down *GATA2* to 50% or less in the HSC compartment in an attempt to recapitulate the effect of a *GATA2* heterozygous phenotype. The best of six shRNAs tested was used for subsequent experiments:
Forward:CCGGTGTGCAAATTGTCAGACGACAACTCGAGTTGTCGTCTGACAATTTG CACTTTTTG
Reverse:AATTCAAAAAGTGCAAATTGTCAGACGACAACTCGAGTTGTCGTCTGACA ATTTGCACA

Lineage-negative (Lin-) CD34+CD38- cord blood (CB) cells from 2 independent pools of CB were transduced with lentiviral constructs expressing GATA2-short hairpin RNA (shRNA) or LacZ-shRNA . After 48h, Lin-CD34+CD38-CD90+ GFP+ or BFP+ hematopoietic stem cells (HSC) were sorted and equal number of GATA2-shRNA induced and control cells transplanted into the right femur of mice irradiated with 225 rads 24 h beforehand. Cells were harvested after 8 weeks and analyzed for surface markers by flow cytometry. The development of CD19+ B cells, CD33+ myeloid cells, common myeloid progenitors (CD34+CD38+CD45RA-CD135+) and MLP (CD34+CD38-CD45RA+) to compare the competitive performance of HSC transduced by *GATA2* shRNA compared with control LacZ shRNA.

A: Efficiency of *GATA2* knock-down in primary lineage-negative cord blood cells. RNA was prepared from sorted GFP+ progenitor cells and relative GATA2 expression measured by RT-PCR. LacZ: LacZ control; G2shRNA: *GATA2* short hairpin RNA. B: The frequency of progenitors in competitive repopulation assays with *GATA2* short hairpin RNA transduced human cord blood HSC compared with control LacZ transduced HSC after 8 weeks xenografting in NSG mice. Left: myeloid progenitors (CD34+CD38+CD135+CD45RA-) and right: multilymphoid progenitors (CD34+CD38-CD45RA+). LG: LacZ-shRNA GFP; LB: LacZ-shRNA BFP; G2G: GATA2-shRNA GFP. C: Representative flow cytometry plots of xenografts in NSG mice bearing LacZ-shRNA BFP or GATA2-shRNA GFP transfected HSC showing CD19+ B cells, CD33+ myeloid cells and the primitive CD34+CD38- progenitor compartment. There was no significant effect of *GATA2* knock-down in the generation of hematopoietic progenitors or balance of lymphoid/myeloid output.

Supplementary Figure 2



Supplementary Figure 3. ***Other biomarkers of GATA2 mutation***

Comparison of stromal growth factors EGF and FGF-2 and serum GM-CSF and CD40L in controls, *GATA2* mutation and MDS. Data shown were derived independently of the multiplex screening assay, using a dedicated ELISA for each marker and a larger collection of serum from *GATA2* patients * $P<0.05$; ** $P<0.01$.