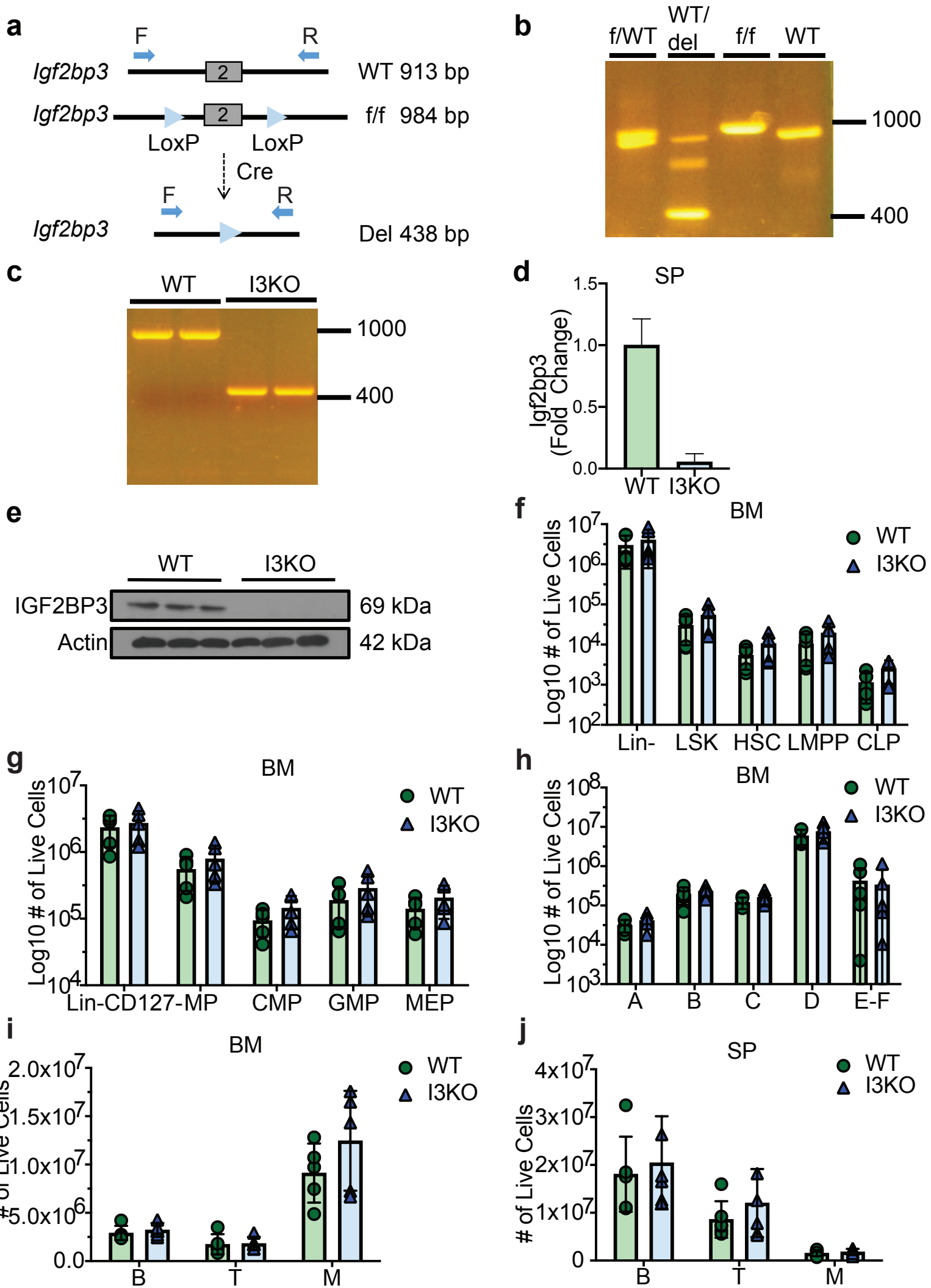


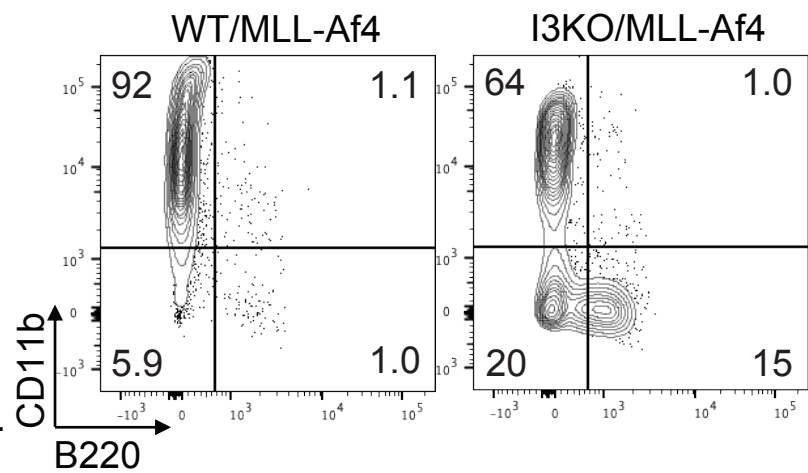
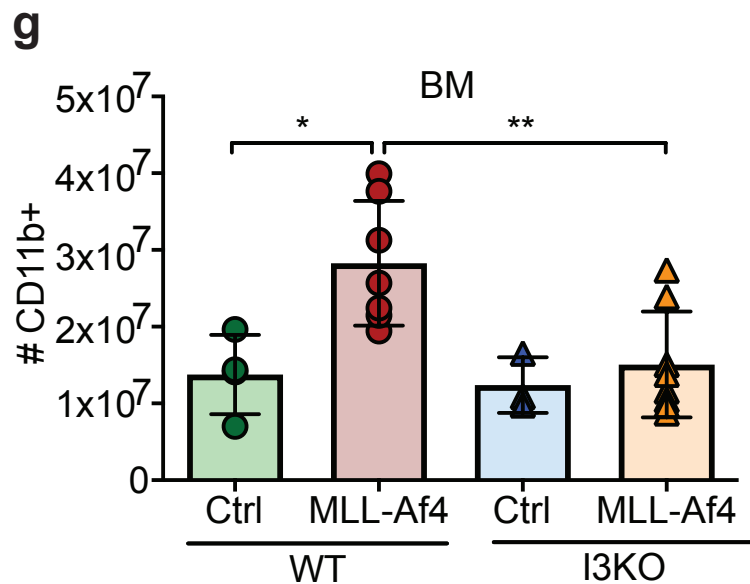
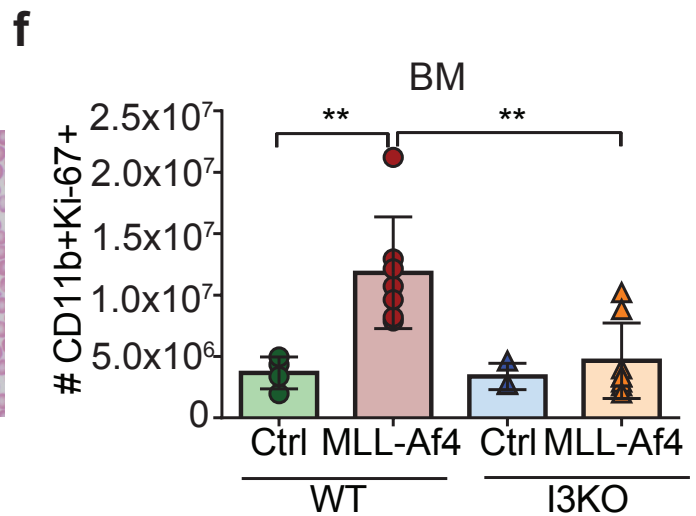
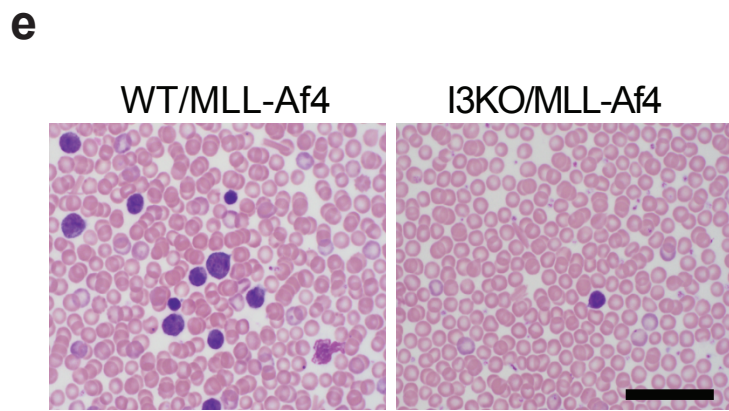
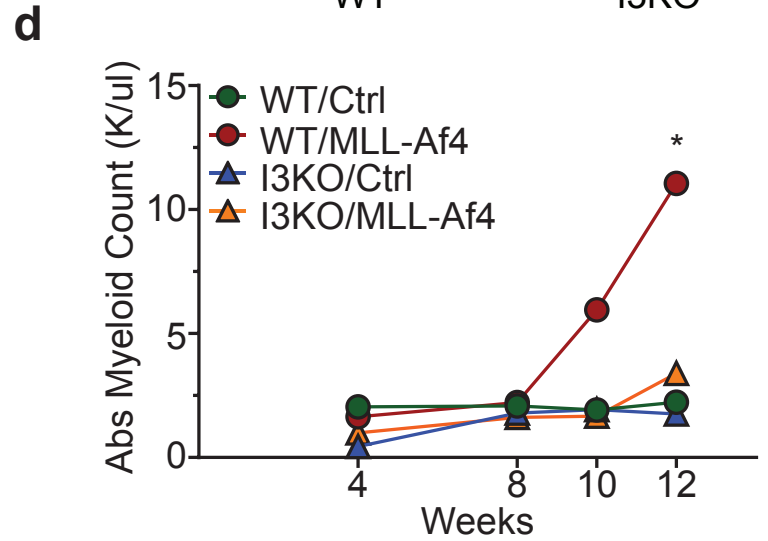
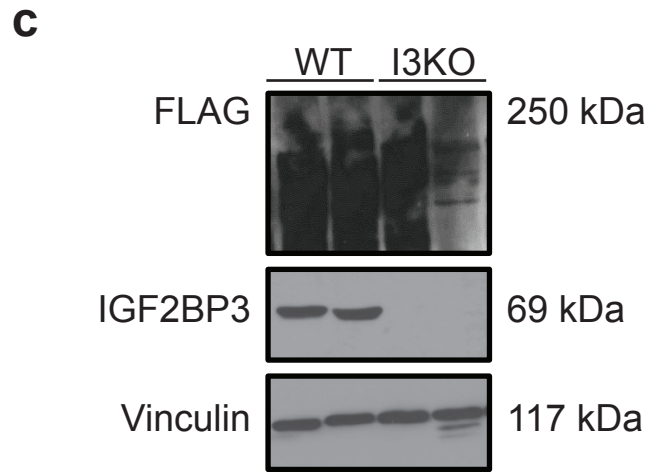
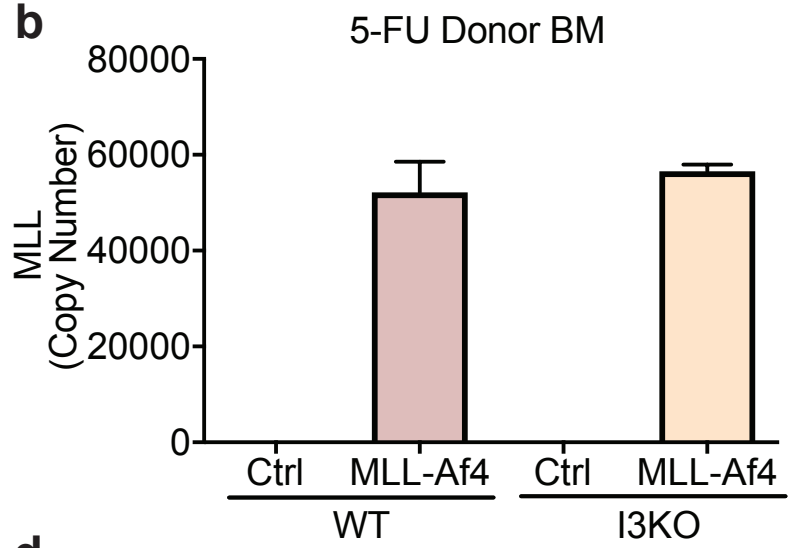
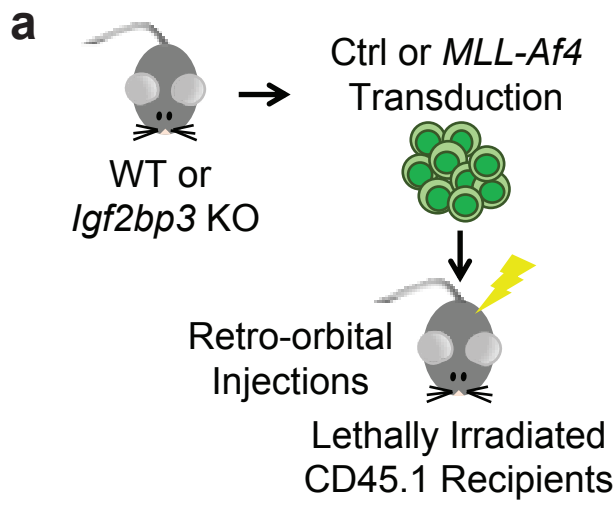
Supplemental Figure 1, Related to Figure 1. IGF2BP3 binds to MLL-AF4 transcriptional target genes and MLL-AF4 induces IGF2BP3.

- a) UCSC Genome Browser snapshot of RS4;11 IGF2BP3 CLIP-seq target MYC, an MLL-AF4 target gene.
- b) UCSC Genome Browser snapshot of CD19+ MLL-Af4 FLAG ChIP-seq target IGF2BP3 from Lin et al, 2016.
- c) ChIP-qPCR shows fold enrichment for IGF2BP3 and CDKN1B with MLL and AF4 IP in SEM. Normalized to UNTR20, an untranscribed region (t-test; *P < 0.05, **P < 0.01).
- d) Percent input from ChIP-qPCR of SEM cells show reduced binding of MLL-AF4 to IGF2BP3 with treatment of IBET-151 (t-test; *** P < 0.001, ****P < 0.0001).
- e) Luciferase assay of the IGF2BP3 promoter shows a dose-dependent response to MLL-AF4.
- f) Induction of *Igf2bp3* at the RNA level in MLL-Af4, MLL-AF9, AML1-ETO, MYC and NRAS Cas9-GFP HSPCs.
- g) Induction of *Igf2bp1* at the RNA level in MLL-Af4, MLL-AF9, AML1-ETO, MYC and NRAS Cas9-GFP HSPCs.
- h) Induction of *Igf2bp2* at the RNA level in MLL-Af4, MLL-AF9, AML1-ETO, MYC and NRAS Cas9-GFP HSPCs.



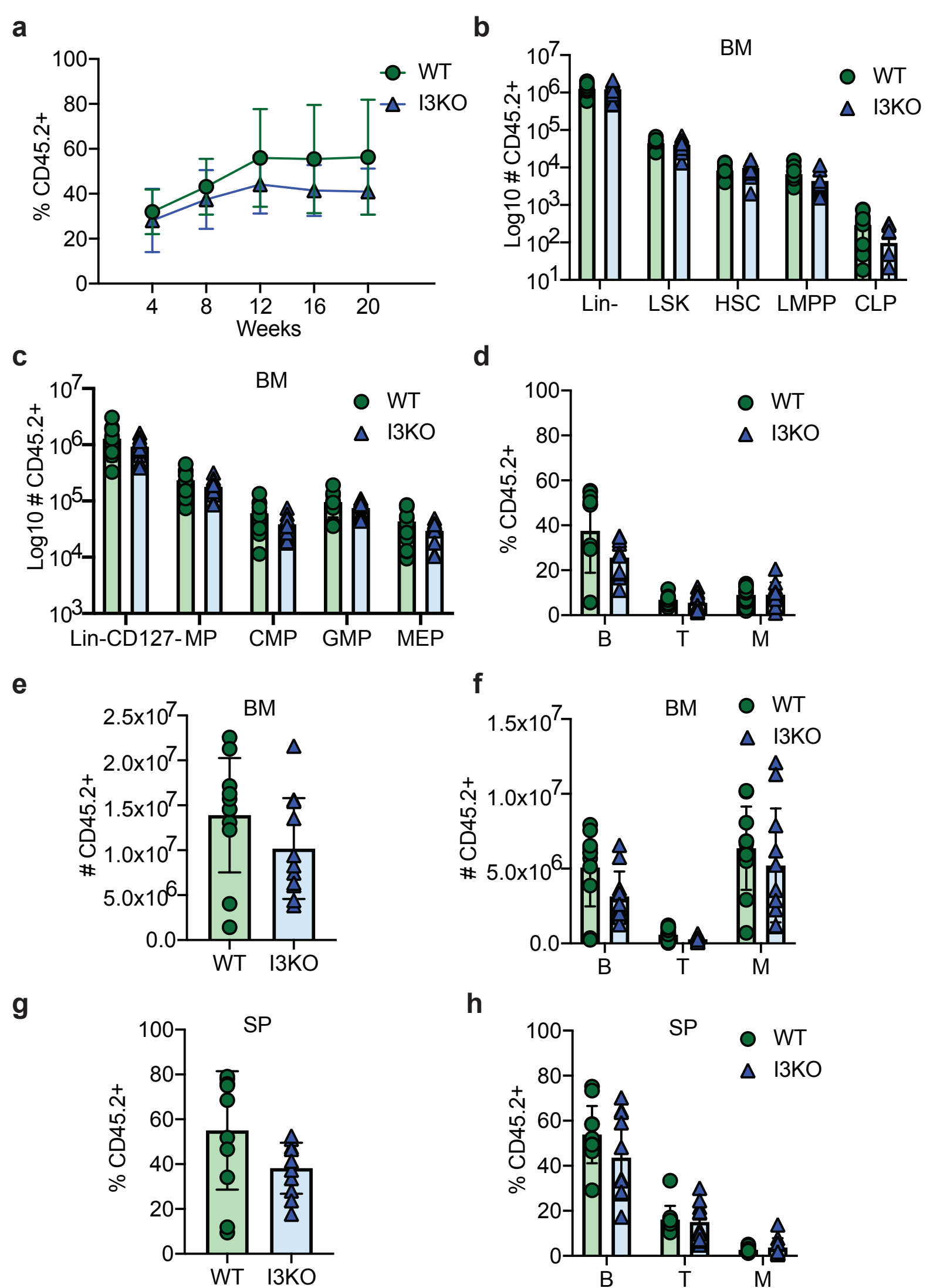
Supplemental Figure 2, Related to Figure 2. I3KO mice maintain normal, steady-state hematopoiesis.

- a) Schematic of alleles in WT, *Igf2bp3^{ff}*, and *Igf2bp3^{del/del}* (I3KO) mice.
- b) PCR genotyping showing WT, *Igf2bp3^{ff}*, heterozygous *Igf2bp3^{ff/WT}* and heterozygous *Igf2bp3^{WT/del}* alleles.
- c) PCR genotyping showing WT and *Igf2bp3^{del/del}* KO alleles.
- d) *Igf2bp3* expression using RT-qPCR in the spleen of I3KO mice.
- e) WB of IGF2BP3 expression in the spleen (tissue with the highest expression of I3) of WT and I3KO mice.
- f) Quantitation of Lin⁻, LSKs, HSCs, LMPPs, and CLPs in the BM of WT and I3KO mice at 8 weeks (n=5 WT, n=5 I3KO; t-test; P = 0.55, 0.23, 0.16, 0.22, 0.09, respectively).
- g) Quantitation of Lin-CD127⁻, Myeloid progenitors (MP), CMPs, GMPs, and MEPs in the BM of WT and I3KO mice at 8 weeks (n=5 WT, n=5 I3KO; t-test; P = 0.67, 0.35, 0.22, 0.34, 0.27, respectively).
- h) Hardy Fractions of WT and I3KO mice at 8 weeks (n=5 WT, n=5 I3KO; t-test; P = 0.30, 0.52, 0.21, 0.44, 0.78, respectively).
- i) Quantification of B cells, T cells, and Myeloid cells in the BM at 8 weeks (n=5 WT, n=5 I3KO; t-test; P = 0.51, 0.88, 0.25, respectively).
- j) Quantification of B cells, T cells, and Myeloid cells in the spleen of WT and I3KO mice at 8 weeks (n=5 WT, n=5 I3KO; t-test; P = 0.70, 0.57, 0.68, respectively).



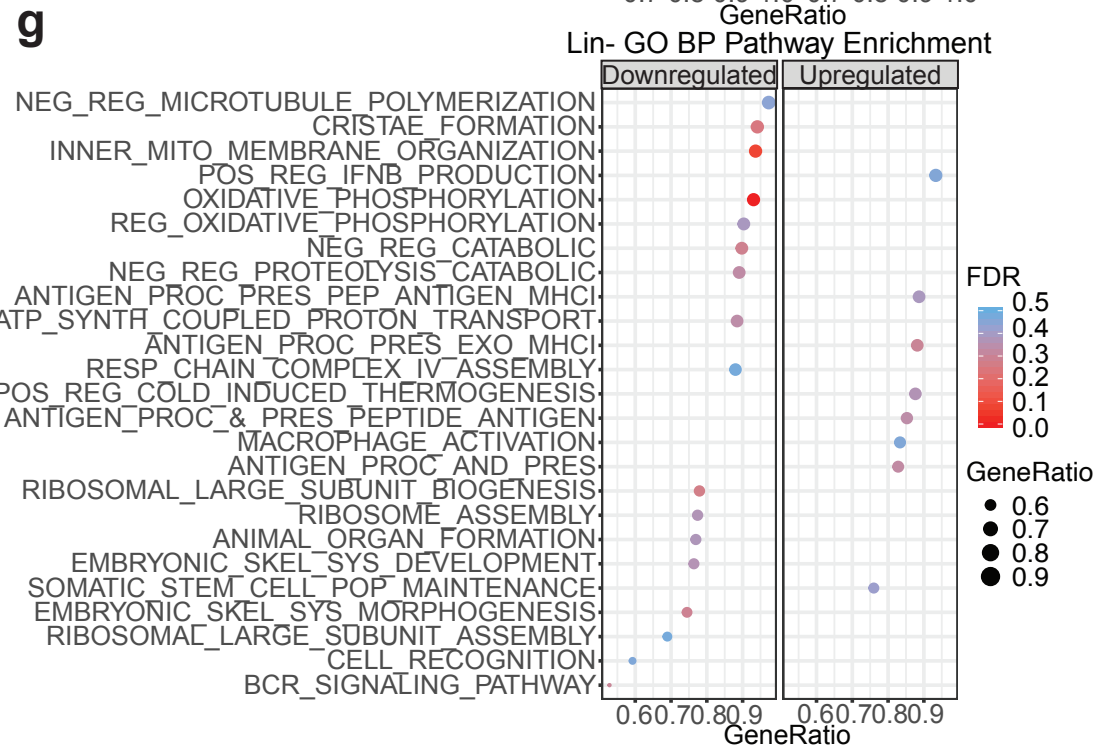
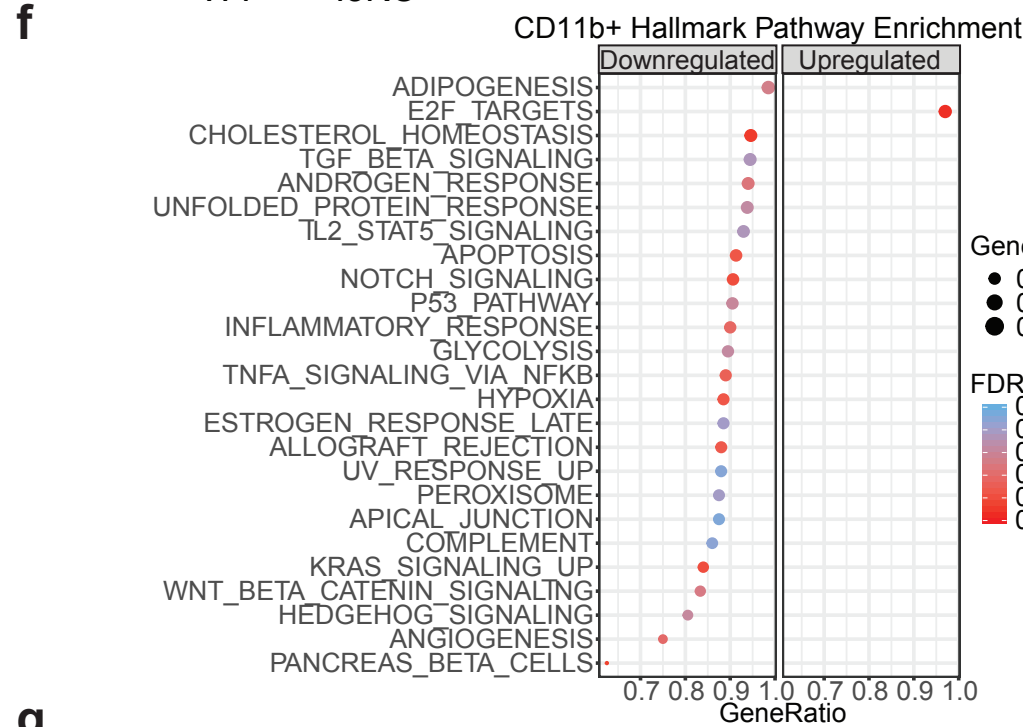
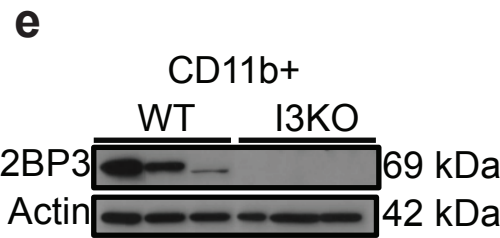
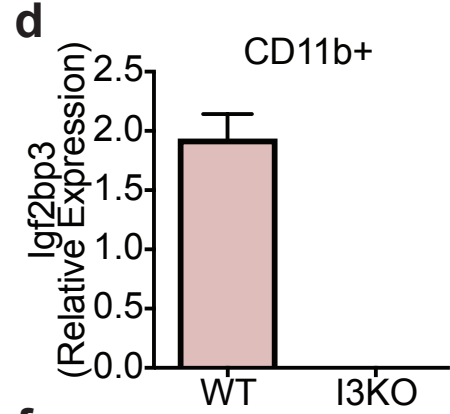
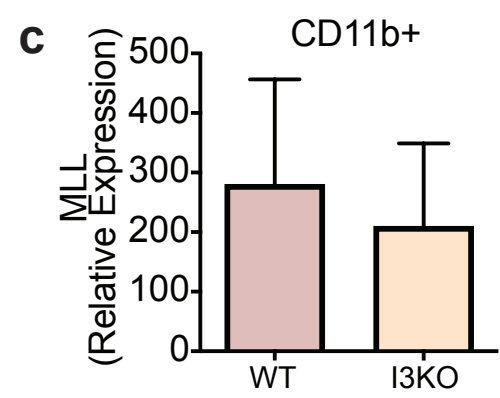
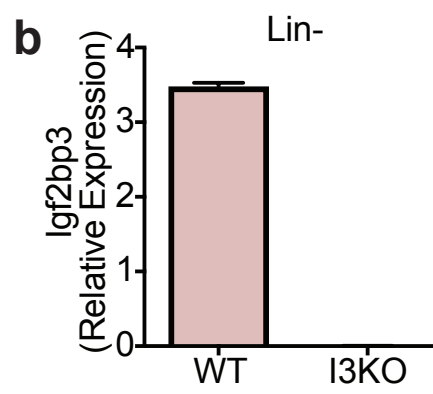
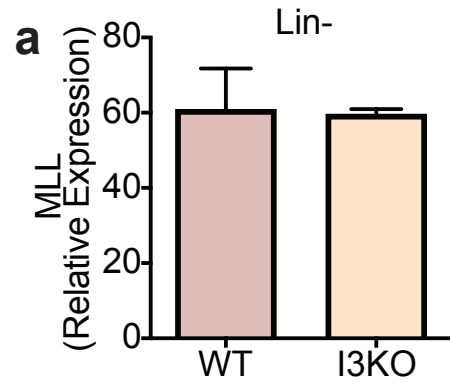
Supplemental Figure 3, Related to Figure 2. *Igf2bp3* deletion attenuates the disease and results in the reduction of leukemic cells.

- a) Schematic of BMT.
- b) MLL-Af4 copy number in 5-FU treated bone marrow from CD45.2 donor mice.
- c) Western blot of BM from mice transplanted with MLL-Af4 transduced WT or I3KO donor HSPCs with antibodies to FLAG, IGF2BP3 and Vinculin.
- d) Time course of Absolute Myeloid Counts in the PB of mice transplanted with control (Ctrl) or MLL-Af4 transduced HSPCs from WT or I3KO mice (t-test; *P < 0.05).
- e) Wright staining of PB smears from WT/MLL-Af4 and I3KO/MLL-Af4 mouse. Scale bar, 40 microns
- f) Quantitation of CD11b+Ki67+ cells in the BM at 14 weeks post-transplantation (n= 4 Ctrl, n=8 MLL-Af4; one-way ANOVA followed by Bonferroni's multiple comparisons test; **P < 0.01).
- g) (Left) Number of CD11b+ in the BM of recipient mice that received Ctrl or MLL-Af4 transduced HSPCs from WT or I3KO mice at 14 weeks (n=4 Ctrl, n=8 MLL-Af4; one-way ANOVA followed by Bonferroni's multiple comparisons test; *P < 0.05, **P < 0.01). (Right) Corresponding representative FACS plots showing CD11b+ and B220+ cells in the BM.



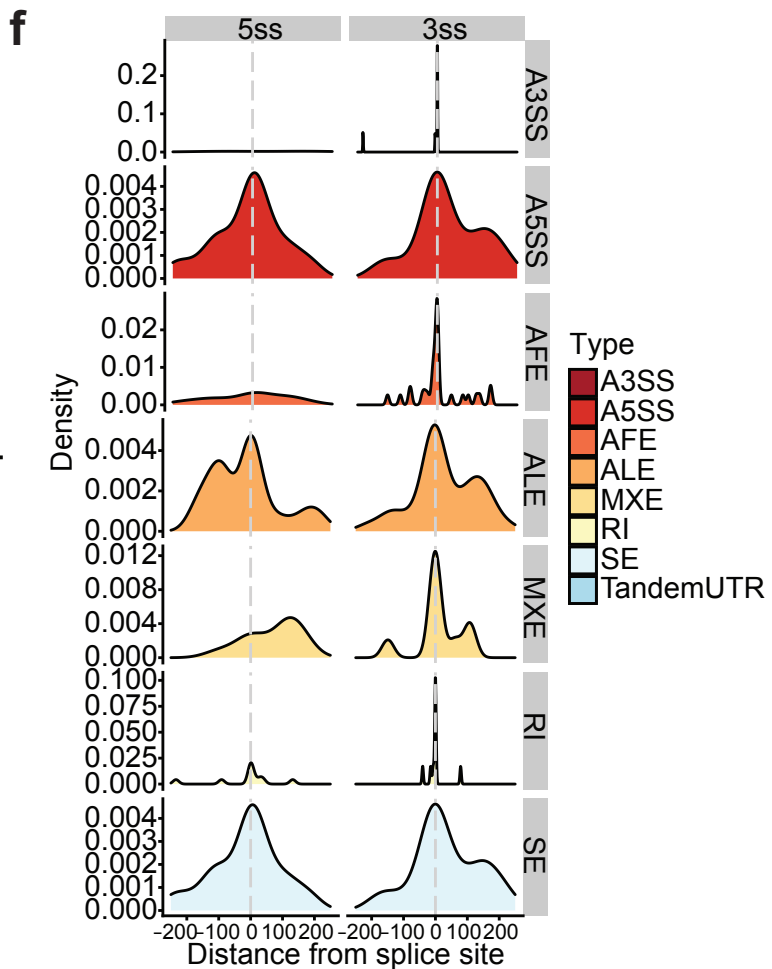
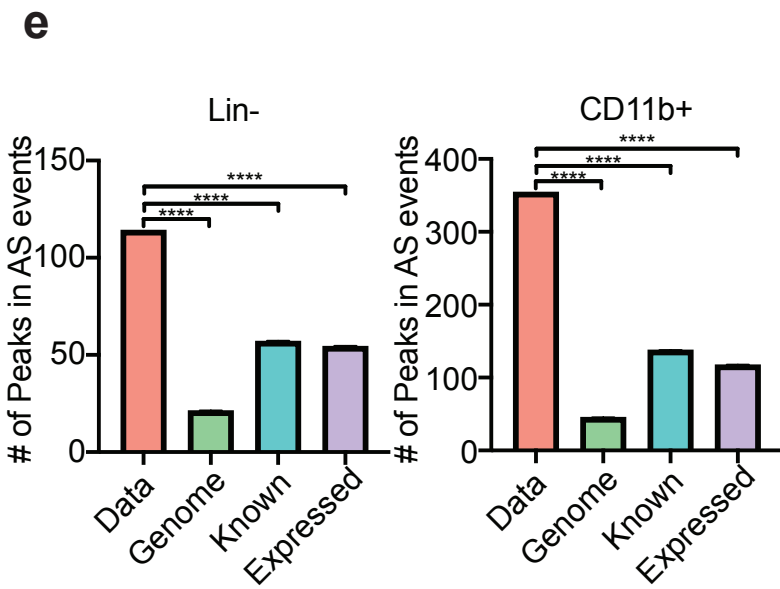
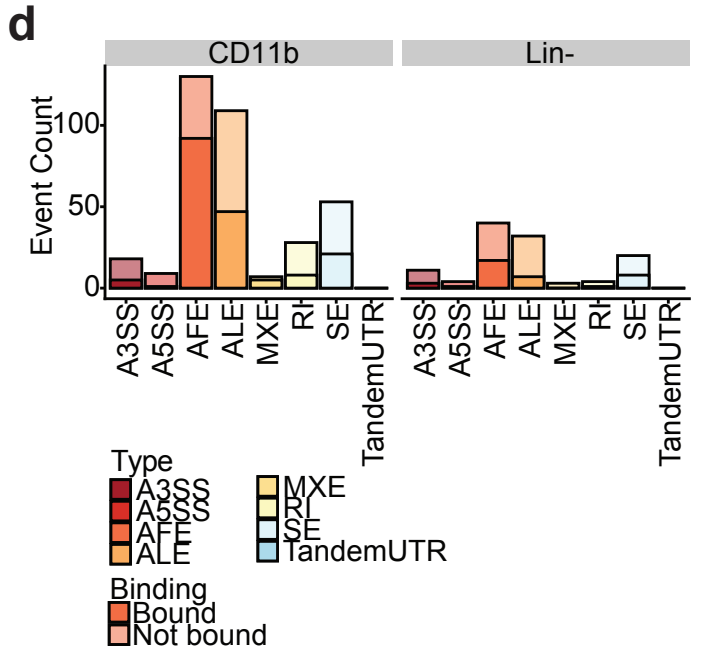
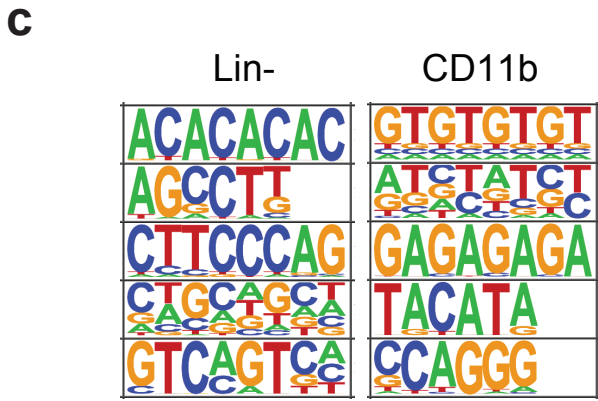
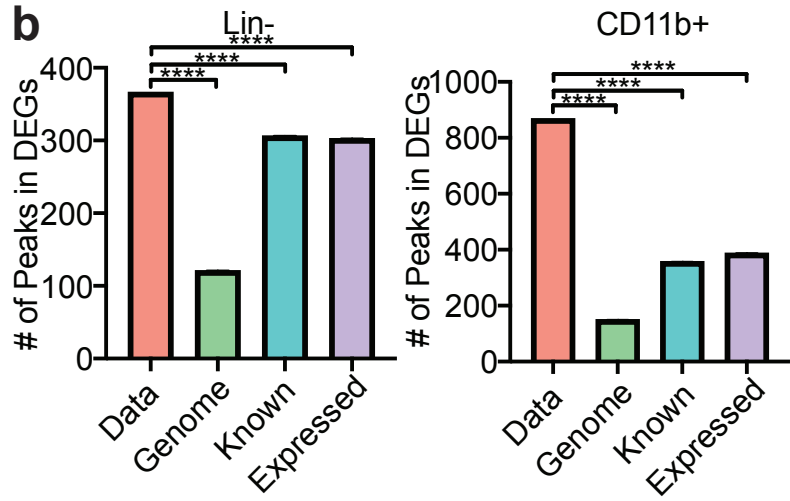
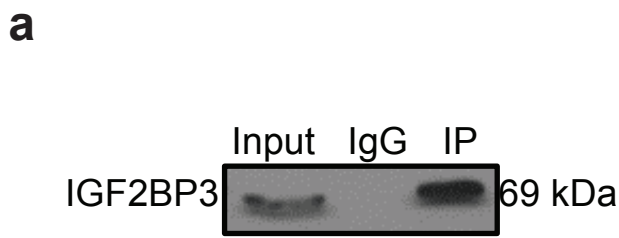
Supplemental Figure 4, Related to Figure 3-4. I3KO mice show normal hematopoietic stem cell function and ability to reconstitute mice.

- a) Percentage of CD45.2+ engraftment in the PB of recipient mice transplanted with either WT or I3KO donor BM in competitive repopulation BMT (n=11 WT, n=11 I3KO; t-test; P = 0.47, 0.31, 0.13, 0.1, 0.08, respectively).
- b) Quantitation of Lin-, LSKs, HSCs, LMPPs, and CLPs in the BM of competitive repopulation BMT mice at 24 weeks post-transplantation (n=11 WT, n=11 I3KO; t-test; P = 0.92, 0.51, 0.42, 0.13, 0.051, respectively).
- c) Quantitation of Lin-CD127-, Myeloid progenitors (MP), CMPs, GMPs, and MEPs in the BM of competitive repopulation BMT at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.17, 0.18, 0.09, 0.18, 0.17, respectively).
- d) Percentage of CD45.2+ B cells (B), T cells (T), Myeloid cells (M) in the PB of competitive repopulation BMT mice at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.07, 0.34, 0.95, respectively).
- e) Quantification of CD45.2+ cells in the BM of competitive repopulation BMT mice at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.16).
- f) Quantification of CD45.2+ B cells (B), T cells (T), Myeloid cells (M) in the BM of competitive repopulation BMT mice at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.051, 0.06, 0.43, respectively).
- g) Percentage of CD45.2+ cells in the SP of competitive repopulation BMT mice at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.07).
- h) Percentage of CD45.2+ B cells (B), T cells (T), Myeloid cells (M) in the SP of competitive repopulation BMT mice at 24 weeks (n=11 WT, n=11 I3KO; t-test; P = 0.14, 0.72, 0.44, respectively).



Supplemental Figure 5, Related to Figure 3 and 5. *Igf2bp3* is upregulated in MLL-Af4 leukemia cells and enhances leukemogenesis by targeting transcripts within Ras signaling pathways.

- a) Relative expression of *MLL* by RT-qPCR in WT/MLL-Af4 and I3KO/MLL-Af4 immortalized Lin⁻ cells.
- b) Relative expression of *Igf2bp3* by RT-qPCR in WT/MLL-Af4 and I3KO/MLL-Af4 immortalized Lin⁻ cells.
- c) Relative expression of *MLL* by RT-qPCR in WT/MLL-Af4 and I3KO/MLL-Af4 leukemic CD11b⁺ splenic cells.
- d) Relative expression of *Igf2bp3* by RT-qPCR in WT/MLL-Af4 and I3KO/MLL-Af4 leukemic CD11b⁺ splenic cells.
- e) Expression of IGF2BP3 in WT/MLL-Af4 and I3KO/MLL-Af4 leukemic CD11b⁺ splenic cells at the protein level.
- f) Hallmark Pathway enrichment using Gene Set Enrichment Analysis on DEseq analyzed RNA-seq samples from WT/MLL-Af4 and I3KO/MLL-Af4 CD11b⁺ cells.
- g) Gene Ontology Biological Processes Pathway enrichment using Gene Set Enrichment Analysis on DEseq analyzed RNA-seq samples from WT/MLL-Af4 and I3KO/MLL-Af4 Lin⁻ cells.



Supplemental Figure 6, Related to Figure 6. *Igf2bp3* targets specific mRNA regulons through multiple post-transcriptional mechanisms.

- a) Confirmation Western blot of protein samples from IGF2BP3 RIP. Input refers to 70Z/3 cell lysate used for immunoprecipitation. RIP is RNA immunoprecipitation from control (mouse IgG) or α -IGF2BP3 antibody used in eCLIP (MBL).
- b) Number of predicted eCLIP binding sites within the differentially expressed genes in the Lin- (left) and CD11b+ (right) dataset, the whole genome, known genes, and expressed genes by simulating distribution of binding sites over respective background (data represent mean \pm SEM; one-sample t-test; ****P < 2.2x10⁻¹⁶).
- c) Homer motif analysis showing top five 6-8-mer motifs.
- d) Event counts for different types of alternative splicing patterns in CD11b and Lin- datasets (A3SS, Alternative 3' splice sites; A5SS, Alternative 5' splice sites; AFE, Alternative first exons; ALE, Alternative last exons; MXE, Mutually exclusive exons; RI, Retained introns; SE, Skipped exons; Bound, IGF2BP3 eCLIP target; Bayes factor \geq 10, delta PSI \geq 0.1, and minimum 20 reads supporting the event).
- e) Number of predicted eCLIP binding sites within the dynamic alternative splicing events in the Lin- (left) and CD11b+ dataset (right), the whole genome, known genes, and expressed genes by simulating distribution of binding sites over respective background (data represent mean \pm SEM; one-sample t-test; ****P < 2.2x10⁻¹⁶).
- f) Histogram showing alternative event density and distance from 5' (5ss) and 3' (3ss) splice sites for each alternative splicing pattern (A3SS, Alternative 3' splice sites; A5SS, Alternative 5' splice sites; AFE, Alternative first exons; ALE, Alternative last exons; MXE, Mutually exclusive exons; RI, Retained introns; SE, Skipped exons).

Table S1, Related to Methods. Antibodies and oligonucleotides used in experiments.

REAGENT	VENDOR/REFERENCE	CATALOG NUMBER
Antibodies		
MLL rabbit polyclonal	Bethyl Lab	A300-086A
AF4 rabbit polyclonal	Abcam	ab31812
RNA Polymerase mouse monoclonal	EMD Millipore	05-623B
Normal Mouse IgG	EMD Millipore	12-371B
FLAG mouse monoclonal	Sigma-Aldrich	F1804
IGF2BP3 rabbit polyclonal	MBL	RN009P
Vinculin mouse monoclonal	Santa Cruz Biotechnology	sc-73614
β -actin mouse monoclonal	Sigma-Aldrich	A1978
HOXA9 goat polyclonal	Santa Cruz Biotechnology	sc-17155
CD3e-PE	Biolegend	100308
CD11b-PE-Cy7	Biolegend	101216
B220-PercP-Cy5.5	Biolegend	103236
Gr-1-Pacific Blue	Biolegend	108430
CD45.1-APC-Cy7	Biolegend	110716
CD45.2-APC	Biolegend	109813
c-Kit/CD117-Alexa Fluor 700	Thermo Fisher Scientific	56-1172-80
Ki-67-PE	Biolegend	652403
c-Kit/CD117-APC-Cy7	Biolegend	105826
Sca1-PerCP-Cy5.5	Biolegend	108124
CD135- APC	Thermo Fisher Scientific	17-1351-82
CD127-PE-Cy7	Biolegend	135014
CD150-PE	Biolegend	115903
Biotin CD4	Thermo Fisher Scientific	13-0041-82
Biotin CD8	Biolegend	100704
Biotin B220	Biolegend	103204
Biotin NK1.1	Biolegend	108704
Biotin IgM	Thermo Fisher Scientific	13-5790-85
Biotin Gr-1	Biolegend	108404
Biotin Ter119	Biolegend	116204
Biotin TCR beta	Biolegend	109204
Biotin TCR gamma-delta	Biolegend	118103
CD45.2-BV605	Biolegend	109841
Streptavidin-Pacific Blue	Thermo Fisher Scientific	48-4317-82
CD16/32-PE-Cy7	Biolegend	101318
CD34-Alexa Fluor 700	Thermo Fisher Scientific	56-0341-82
CD127-APC	Biolegend	135012
IgM-PE	SoutherBiotech	1020-09S
CD43- APC	BD Biosciences	560663
CD24-PE-Cy7	Thermo Fisher Scientific	25-0242-80
Ly51-Biotin	Biolegend	108303

Oligonucleotides		
IGF2BP3-ChIP F: GACCACGAACGGGAGAACTG R: TCAATTCAGACGTGGTGCGG	Lin et al., 2016.	N/A
CDKN1B-ChIP F: TCTTCTTCGTCAGCCTCCCTTC R: TCGCAGAGCCGTGAGCAAGC	Wilkinson et al., 2013.	N/A
MLL F: GAAACCTACCCCATCAGCAA R: GACCTGCTTGCTTGACTTCC	This paper	N/A
Igf2bp3 F: CCTGGTGAAGACGGGCTAC R: TCAACTTCCATCGGTTTCCCA	This paper	N/A
Igf2bp1 F: CTC AACGAGAGTGTGACCCC R: CCCGAGAAAGTTTCGATGGC	This paper	N/A
Igf2bp2 F: TAAGGCTCAGGGACGGAT R: TGGTCACGAGGCACGATAAC	This paper	N/A
Hoxa7 F: ATGTGAACGCGCTTTTTAGC R: ATTGTATAAGCCCGGCACAG	This paper	N/A
Hoxa9 F: AAAACACCAGACGCTGGAAC R: TCTTTTGCTCGGTCCTTGTT	This paper	N/A
Hoxa10 F: GAAGAAACGCTGCCCTTACA R: GATTCGGTTTTCTCGGTTCA	This paper	N/A
Cd69 F: TGGTGAAC TGG AACATTGGA R: CTCACAGTCCACAGCGGTAA	This paper	N/A
Notch1 F: TGAGACTGCCAAAGTGTGTC R: GTGGGAGACAGAGTGGGTGT	This paper	N/A
Csf2rb F: CCCACTCTCTGCCTGATCTC R: CCCACACTGCACATCCATAG	This paper	N/A
Ccnd1 F: GCGTACCCTGACACCAATCT R: CTCTTCGCACTTCTGCTCCT	This paper	N/A
Maf F: GAGGAGGTGATCCGACTGAA R: TCTCCTGCTTGAGGTGGTCT	This paper	N/A
Itga6 F: TGAAGATGGGCCCTATGAAG R: CTCTTGGAGCACCAGACACA	This paper	N/A
Mafb F: TGGATGGCGAGCAACTACC R: CCAGGTCATCGTGAGTCACA	This paper	N/A
Klf4 F: CTGAACAGCAGGGACTGTCA R: GACCTTCTTCCCCTCTTTGG	This paper	N/A
Akt3 F: GCCCAACCTCACAGATTGAT R: CACTTGCCTTCTCTCGAACC	This paper	N/A
Hoxa9T F: CCGGACGGCAGTTGATAG R: GTCCAGCGTCTGGTGT	This paper	N/A
Hoxa9-FL F: ATCACCACCACCCTAC R: GAGTGTCAAGCGTGGGACAG	This paper	N/A
Hoxa9-Common F: CCTGGGCAACTACTATGTGGA R: GGAAGCTGCAAGGACTGAAG	This paper	N/A
Hoxa7-Alt F: GCTCGGCTAGAGGACACC R: TGATAGAGGGGCTGTTGAC	This paper	N/A
Hoxa7-FL F: ATGTGAACGCGCTTTTTAGC R: ATTGTATAAGCCCGGCACAG	This paper	N/A
Hoxa7-Common F: GCCTCCTACGACCAAAACAT R: GTCTGGTAGCGGTGTAGGT	This paper	N/A
Cd69-Alt F: AGAGGGCAGAAGGTGGGC R: CAAGGCCCAACTCTTGTT	This paper	N/A
Cd69-FL F: GTGGTCTCATCACGTCCTT R: CAACTCTTGTTGTGGTGGGA	This paper	N/A
Cd69-Common F: TGGTGAAC TGG AACATTGGA	This paper	N/A

R: CTCACAGTCCACAGCGGTAA		
Igf2bp3 sgRNA F:CACCGAGCTTGGTCCTTACTGGAAT R:AAACATTCCAGTAAGGACCAAGCTC	Palanichamy et al., 2016.	N/A
Non-targeting sgRNA F:TTTGCGAGGTATTCGGCTCCGCG R: AAACCGCGGAGCCGAATACCTCG	Sanjana et al., 2014.	N/A
L32 F: AAGCGAAACTGGCGGAAAC R: TAACCGATGTTGGGCATCAG	Palanichamy et al., 2016.	N/A
UNTR20 F: ATCACACTGCAAAAATCCAGAA R: TCACTTCTTTAACTGGCCTTGA	Kaukonen et al., 2016.	N/A

Table S2, Related to Supplemental Figure 2. Chi-square of generation of *Igf2bp3*^{del/del} (I3 KO) mice.

Genotype	Observed (O)	Expected (E)	O-E	(O-E) ²	(O-E) ² /E	χ^2	P value
WT	20	14	6.0	36.0	2.57	4.39	0.1-0.15
WT/del	21	28	-7.0	49.0	1.75		
del/del	15	14	1.0	1.0	0.07		

Table S3, Related to Figure 5-6. IGF2BP3-dependent regulatory targets in MLL-Af4 Lin- cells. Provided as an Excel file.

Table S4, Related to Figure 5-6. IGF2BP3-dependent regulatory targets in MLL-Af4 CD11b+ bulk leukemia cells. Provided as an Excel file.

SUPPLEMENTAL METHODS

ChIP-PCR reagents. RS4;11 and SEM cells were crosslinked using 37% formaldehyde and subjected to sonication to shear the crosslinked chromatin. Chromatin immunoprecipitation was performed using the EZ-ChIP kit (EMD Millipore 17-371). Antibodies used were MLL rabbit polyclonal (Bethyl Lab A300-086A), AF4 rabbit polyclonal (Abcam ab31812), RNA Polymerase mouse monoclonal (EMD Millipore 05-623B), and Normal Mouse IgG (EMD Millipore 12-371B). SEM cells were treated with 1 μ M of EPZ5676 (Sigma-Aldrich ADV465750917) and 1 μ M of IBET151 (Sigma-Aldrich SML0666).

qPCR reagents. RNA from cell lines or primary mouse cells was collected using QIAzol Lysis Reagent and purified using the miRNeasy Mini Kit (Qiagen 217004). RNA was reverse transcribed using qScript (Quanta BioSciences). qPCR was performed with the StepOne Plus Real-Time PCR System (Applied Biosystems) using PerfeCTa SYBR Green FastMix reagent (Quanta BioSciences).

Western blot reagents. Cells were lysed in RIPA buffer (Boston BioProducts) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific). Equal amounts of protein lysate (as quantified by using bicinchoninic acid protein assay, BCA [Thermo Scientific]) were electrophoresed on a 5%–12% SDS-PAGE and electroblotted onto a nitrocellulose membrane. Antibodies used were FLAG mouse monoclonal (Sigma-Aldrich F1804), IGF2BP3 rabbit polyclonal (MBL RN009P), MLL rabbit polyclonal (Bethyl Lab A300-086A), HOXA9 goat polyclonal (Santa Cruz Biotechnology Inc. sc-17155), Vinculin mouse monoclonal (Santa Cruz Biotechnology Inc. sc-73614) and β -actin mouse monoclonal (Sigma-Aldrich A1978). Secondary HRP-conjugated antibodies (Santa Cruz Biotechnology Inc.) and SuperSignal West Pico Plus kit (Pierce Biotechnology) were used for enhanced chemiluminescence-based detection. Quantification was performed using ImageJ analysis.

Luciferase assays. The 950bp promoter region upstream of the TSS of IGF2BP3 was cloned into the multiple cloning region between NheI and HindIII restriction enzyme sites of the pGL4.11 vector (Promega). 293T cells were cotransfected with the pGL4.11, pGL4.75, IGF2BP3 promoter containing reporter vector along with the empty vector or PIDE-MLL-AF4 overexpression vector ratios (0:400, 375:25, 350:50, 300:100, 200:200, and 0:400 ng). Cotransfections were performed with BioT (Bioland) as per the manufacturer's instructions. Cells were lysed after 24 hours, substrate was added, and luminescence was measured on a GloMax-Multi Jr (Promega) utilizing the Dual-Luciferase Reporter Assay System (Promega E1910). The ratio of firefly to Renilla luciferase activity was calculated for all samples. Experiments were completed at least 3 times for validation.

Additional plasmids, cell culture, and spin infection details. The PIDE-MLL-AF4 plasmid used in the luciferase assay was kindly given by Rolf Marschalek (Goethe-University of Frankfurt, Frankfurt, Germany) through MTA(1). The MIG-MLL-AF9 plasmid was kindly given by John Chute (Cedars-Sinai Medical Center, Los Angeles, CA). The MigR1-AE vector encoding the AML1-ETO fusion was purchased from addgene (addgene 12431). The coding sequences for MYC and NRAS were cloned in-house into the MSCV-IRES-

GFP (MIG) vector, respectively. 70Z/3 cells (ATCC TIB-158) were spin-infected at 30°C for 90 minutes in the presence of polybrene. Lin⁻ cells were obtained by harvesting the BM from C57BL/6J (Jackson Laboratory 000664) or I3KO mice, lysing in red blood cell lysis buffer, and staining with a cocktail of biotinylated antibodies for lineage depletion by MACS (Miltenyi). Cas9 Lin⁻ cells were obtained by harvesting the BM from B6J.129(Cg)-Gt(ROSA)26Sor^{tm1.1(CAG-cas9*,-EGFP)Fezh/J} mice (Jackson Laboratory 026179), lysing in red blood cell lysis buffer, and staining with a cocktail of biotinylated antibodies for lineage depletion by MACS (Miltenyi). Cas9 Lin⁻ cells were spin-infected with MIG-MLL-AF9, MigR1-AE, MIG-MYC, and MIG-NRAS virus at 30°C for 90 minutes in the presence of polybrene. For CFU assays, after MLL-Af4 transduction and G418 selection, Cas9 Lin⁻ cells were spin-infected with MSCV-hU6-sgRNA-EFS-mCherry virus at 30°C for 90 minutes in the presence of polybrene. CD11b⁺ cells were obtained by harvesting the splenic tumors from the WT/MLL-Af4 or I3KO/MLL-Af4 mice, lysing in red blood cell lysis buffer, and staining with a CD11b biotinylated antibody (BioLegend 101204) for positive selection by MACS (Miltenyi). The list of antibodies used is provided in the Table S1. The human B-ALL cell lines RS4;11 (MLL-AF4 translocated; ATCC CRL-1873), SEM (MLL-AF4 translocated; DSMZ ACC 546), murine pre-B leukemic cell line 70Z/3 (ATCC TIB-158), and HEK 293T cell line (ATCC CRL-11268) were grown in their corresponding media at 37°C in a 5% CO₂ incubator. Lentiviruses and retroviruses were generated as previously described(2, 3).

Additional BM transplant and competitive repopulation assay details. 5-FU enriched BM was harvested and spin-infected from 8-week-old CD45.2⁺ donor C57BL/6J (Jackson Laboratory 000664) or I3KO female mice as previously described(2). 8-week-old CD45.1⁺ recipient B6.SJL-Ptprc-Pep3/BoyJ (Jackson Laboratory 002014) female mice were lethally irradiated and injected with donor BM. Total sample size (n) of 24 mice (n=4 Ctrl and n=8 MLL-Af4 for WT and I3KO) were used for each experiment. Experiments were completed at least 3 times for validation. For competitive repopulation experiments, 8-week-old CD45.2⁺ donor C57BL/6J or I3KO female mice and 8-week-old CD45.1⁺ donor B6.SJL-Ptprc-Pep3/BoyJ female mice were harvested for BM. CD45.1⁺ and CD45.2⁺ BM cells were mixed in a ratio of 1:1 and injected into lethally irradiated 8-week-old CD45.1⁺ recipient B6.SJL-Ptprc-Pep3/BoyJ female mice. Mice were bled at 4, 8, 10, 12, 14, 16, and 20 weeks after BM injection for analysis of the peripheral blood. For serial secondary transplantation assays, WT/MLL-Af4 or I3KO/MLL-Af4 mice that succumbed to leukemia at 10-14 weeks post-transplantation were euthanized and the BM collected. For each group, 10⁶, 10⁵, or 10⁴ donor BM cells were injected per mouse into 8-week-old immunocompetent CD45.1⁺ recipient B6.SJL-Ptprc-Pep3/BoyJ female mice. Mice were bled at 2 and 4 weeks after BM injection for analysis of the peripheral blood. Sample size are as follows: n=6 10⁶, n=10 10⁵, and n=10 10⁴ for WT and I3KO. Experiments were completed at least 3 times for validation. All mice were purchased from the Jackson Laboratory and housed under pathogen-free conditions at UCLA.

Colony formation assays. WT and I3KO MLL-Af4 Lin⁻ cells were sorted as described previously and 100,000 cells were plated on M3434 (MethoCult) methylcellulose medium containing SCF, IL-3, IL-6, and EPO. Following G418 selection, 5,000 MLL-Af4 expressing WT or I3KO Cas9-GFP Lin⁻ cells were plated on M3434 methylcellulose

medium. Colonies were counted at 7 and 14 days after plating. Experiments were completed at least 3 times for validation.

Flow cytometry staining. Blood, BM, thymus, and spleen were collected from the mice under sterile conditions at 14 weeks post-transplant, 24 weeks post-transplant, or when mice showed pre-moribund status. Single cell suspensions were lysed in red blood cell lysis buffer. Fluorochrome-conjugated antibodies were used for staining. For intracellular staining, after initial staining with surface marker antibodies and fixation with IC Fixation Buffer (Thermo Scientific) cells were incubated with antibodies against intracellular antigens Ki67 (BioLegend 652403) with 1X Permeabilization Buffer (Thermo Fisher Scientific). After 30 minutes of staining at RT, cells were washed twice with Permeabilization Buffer and fixed with 1% PFA. Flow cytometry was performed at the Eli and Edythe Broad Center of Regenerative Medicine and Stem Cell Research UCLA Flow Cytometry Core and the UCLA JCCC Flow Core on a BD FACS LSR II instrument. Analysis was performed using FlowJo software.

eCLIP Library Preparation. Following immunoprecipitation, two percent of each sample was then removed for size matched input libraries. Immunoprecipitated IGF2BP3 and crosslinked RNA were run on and excised from an acrylamide gel. Library preparation was performed as outlined in the Eclipse BioInnovations Inc eCLIP kit (Eclipse BioInnovations ECEK-0001) and final libraries were excised from an acrylamide gel.

eCLIP statistical details. Peaks were removed if they overlapped a minimal amount of peak length (0.0001). Peaks were further filtered for a p -value < 0.05 . Motif analysis was conducted for 4-6 and 6-8bp long motifs over the HOMER standard shuffled background. Distances from peaks to splice sites were determined using bedtools closest.

RNA seq library preparation. The Agilent 2100 Bioanalyzer using RNA pico chip was utilized to quality check total RNA. The Universal plus mRNA-Seq Kit (NuGEN Technologies 0520-24) was used to generate strand-specific RNA-seq libraries. Samples were subjected to poly(A) RNA selection, RNA fragmentation and double-stranded cDNA generation using a mixture of random and oligo(dT) priming. The cDNA samples were then subjected to end repair to generate blunt ends, adapter ligation, strand selection, and PCR amplification to generate the final library. To multiplex samples in one sequencing lane, different index adapters were used.

MISO annotation. The MISO event database for pairwise alternative splicing events for mm10 ("exon-centric annotation") was downloaded from hollywood.mit.edu/burgelab/miso/annotations/. MISO estimates expression level for each annotated event, which is quantified as the percent spliced in (PSI). Delta PSI was calculated by subtracting PSI of the I3KO sample from WT for each event. Significant differential splicing events were filtered between WT and I3KO samples by requiring that $\Delta \text{PSI} > 0.1$, Bayes factor ≥ 10 , and the sum of exclusion and inclusion reads ≥ 10 .

Survival data analysis. Survival data was computed using the Kaplan-Meier method and survival curves were compared using a Log-rank test on GraphPad Prism software. A total sample size of 24 mice will have $> 80\%$ power to detect a hazard ratio of 0.16,

assuming the proportion of the control group without leukemia is 8%, using a two-sided Log-rank test with significance level at 0.01.

Significance threshold. A P value less than 0.05 was considered significant. *P < 0.05, **P < 0.01, ***P < 0.001, and ****P < 0.0001.

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