Supplemental Figure Legends.

Supplemental Figure S1. IGF2BP3 knockdown in human B-ALL cell lines and murine MLL-Af4 Lin- HSPCs decreases cell growth.

(A) Cell viability of SEM, RS4;11 and NALM6 cells depleted for IGF2BP3 (I3KO) or non-depleted (NT) was measured using CellTiterGlo, at multiple timepoints over 120 hours, and is plotted as fold change from D0, mean ± SD, n =6.

(B) Schematic for generation of MLL-Af4 transformed HSPCs depleted for IGF2BP3 using CRISPR/Cas9 by harvesting HSPCs from bone marrow of Cas9-GFP mice, followed by MLL-Af4 transduction, and then retroviral transduction to introduce sgRNA targeting *Igf2bp3* or non-targeting guides.

(C) Cell viability of MLL-Af4 Lin- cells depleted for IGF2BP3 (I3KO) or non-depleted (NT) was measured using CellTiterGlo, at multiple timepoints over 120 hours, and is plotted as fold change from D0, mean ± SD, n =6.

(D) Total colony number is reduced with IGF2BP3 knockdown in MLL-Af4 Lin- cells in methylcellulose colony formation assays. MLL-Af4 Lin- depleted for IGF2BP3 (I3KO) or non-depleted (NT) were seeded at an initial seeding density of 5000 cells, followed by 10 days in methylcellulose culture media. Mean ± SD, n =2 (*t*- test, ** $p < 0.01$).

(E) Ki67 positivity by FACS staining in MLL-Af4 NT and I3KO Lin- cells treated with MI-503 0.5μM or DMSO control, mean ± SD, n =2. (One-way ANOVA with Bonferroni multiple comparisons test)

(F) Total colony number of MI-463 treated MLL-Af4 NT and I3KO Lin- cells is reduced with IGF2BP3 knockdown in methylcellulose colony formation assays. MLL-Af4 Lin- cells depleted for IGF2BP3 (I3KO) or non-depleted (NT) were treated with MI-463 0.5μM or DMSO control for 5 days before seeding 500 cells into methylcellulose culture media for 12 days. Mean ± SD, n =2 (One-way ANOVA with Bonferroni multiple comparisons test, * $p < 0.05$, ** $p < 0.01$).

Supplemental Figure S2. IGF2BP3 depletion, using alternative guides targeting IGF2BP3, show expected changes in downregulation of known IGF2BP3 targets, decreased cell growth, and sensitization to menin-MLL inhibition.

(A) Western blot analysis showing IGF2BP3 knockdown in MLL-Af4 Lin- cells (using I3-sg3 sgRNA targeting *Igf2bp3* and NT-2, as a non-targeting guide).

(B) Expression of *Ig2bp3* and known downstream targets was decreased, as measured by RT-qPCR (mean ± SD, n=2; *t*-test; *p < 0.05, ** p <0.01, ***p< 0.001, **** p< 0.0001).

(C) Cell viability of MLL-Af4 Lin- cells depleted for IGF2BP3 (I3-sg3) and non-depleted (NT-2) was measured using CellTiterGlo, at multiple timepoints over 120 hours, and is plotted as fold change from D0, mean ± SD, n =6.

(D) Dose-response curves from cell viability assays, using CellTiterGlo, of MLL-Af4 NT-2 and I3-sg3 Lin- cells treated with menin-MLL inhibitors, MI-503 and MI-463, for 4 days. Viability has been normalized to DMSO control-treated cells not depleted for IGF2BP3 (NT-2 DMSO), mean ± SD, n = 6.

Supplemental Figure S3. Increased differentiation in MLL-Af4 leukemic cells seen with I3KO and MI-503 by morphology. (A-D) Photomicrographs of Wright stained cytospin preparations of MLL-Af4 Lin- cells: NT, DMSO (A), NT, MI-503 (B), I3KO, DMSO (C) and I3KO, MI-503 treated (D). Arrows point to differentiated cells, such as granulocytic precursors (black arrow), megakaryocytic precursors (green arrow), and macrophage/histiocytic cells (blue arrow). Magnification, 1000x, scale bar: 10μm.

Supplemental Figure S4. Differentially expressed genes in MLL-Af4 Lin- cells with depletion of IGF2BP3 and treatment with menin-MLL inhibitor, MI-503.

(A) Number of differentially expressed genes with MI-503 treatment (0.2 μM or 1.0 μM) vs. DMSO control for 4 days and with IGF2BP3 depletion (I3KO vs. NT) in MLL-Af4 Lin- cells, by DESeq analysis on RNA sequencing samples.

(B) Pathway enrichment for downregulated genes with IGF2BP3 knockdown in MLL-Af4 Lin- cells utilizing Metascape analysis webtool on MLL-Af4 Lin− IGF2BP3 DESeq dataset with an adjusted P < 0.05 cutoff.

(C) Pathway enrichment for downregulated genes with MI-503 treatment (1.0 μM) in MLL-Af4 Lin- NT utilizing Metascape analysis webtool on MLL-Af4 Lin- NT MI-503 DESeq dataset with an adjusted P < 0.05 cutoff.

(D) Pathway enrichment for downregulated (left) and upregulated (right) genes with MI-503 treatment (1.0 μM) in MLL-Af4 Lin- I3KO utilizing Metascape analysis webtool on MLL-Af4 Lin- I3KO MI-503 DESeq dataset with an adjusted P < 0.05 cutoff.

Supplemental Figure S5. IGF2BP3 knockdown via CRISPR/Cas9 leads to decreased leukemic burden *in vivo*.

(A) Schematic of bone marrow transplantation of MLL-Af4 Lin- knocked out for IGF2BP3 (I3KO) vs. control (NT), using CRISPR/Cas9 mediated knockdown.

(B) Decreased leukemic burden in peripheral blood with IGF2BP3 knockdown, based on GFP+mCherry+ cells by FACS at D23, mean ±SD, n = 8 mice/group (*t* test, **** p < 0.0001).

(C-D) Decreased proportion of mice with gross leukemia or pre-leukemia in I3KO mice, in timed sac at D30. Leukemia defined as spleen weight > 150 mg or presence of leukemic blasts in peripheral blood or bone marrow. Pre-leukemia was defined based on morphologic changes seen on histopathology. N = 8 mice/group (Fisher's exact test, *** p < 0.001).

(E-H) Decreased leukemic burden in spleens of I3KO mice, as shown by weights, counts, CD11b+% and mCherry+GFP+%, mean ±SD, n = 8 mice/group (t test, * p < 0.05, *** p < 0.001, **** p < 0.0001).

(I-K) Decreased leukemic burden in bone marrow of I3KO mice, as shown by counts, CD11b+% and mCherry+GFP+%, mean ±SD, n = 8 mice/group (t test, *** p < 0.001, **** p < 0.0001).

Supplemental Figure S6. Additional histology and validation of individual RT-qPCR genes upregulated in differentiation pathways in mice transplanted with MLL-Af4 leukemia cells, depleted for IGF2BP3 and treated with MI-503 *in vitro*

(A) Photomicrographs of H&E stained, formalin fixed, paraffin embedded spleens from mice transplanted with MI-503 treated MLL-Af4 Lin- NT or I3KO cells: left to right, NT DMSO-treated, NT MI-503-treated, I3KO DMSO-treated, and I3KO MI-503 treated.

(B-G) Expression of genes of interest in bone marrow was measured by RT-qPCR. MLL-Af4 Lin-cells depleted (I3KO) or non-depleted (NT) for IGF2BP3 were treated with MI-503 0.5μM (MI-503) or carrier control (DMSO) for 5 days *in vitro* before transplantation. Mice were sacrificed at 8.5 weeks at first signs of first mouse developing terminal leukemia. Gene expression data shown from selected mice in each group. Shown as fold change from NT DMSO (mean ± SD, n=2; one-way ANOVA with Bonferroni's multiple comparisons test ; *p < 0.05, ** p <0.01, ***p< 0.001, **** p< 0.0001).

Supplemental Figure S7. Conditional deletion of *Igf2bp3* in MLL-Af4 leukemia models *in vitro* leads to IGF2BP3 knockdown and expected phenotypic effects but is not inducible in the Mx1-Cre system due to spontaneous deletion.

(A) Schematic of alleles generated in the I3CKO mouse.

(B) Schematic of Mx1-Cre mediated excision of *Igf2bp3* exon 2 following polyI:C induction.

(C) Agarose gel electrophoresis showing genotyping results of DNA from harvested BM of I3CKO or WT mice treated with poly I:C (438 bp band corresponds to deleted allele, and WT is 913bp)

(D) RT-qPCR of bone marrow cells, showing greater than 90% reduction in transcript levels following poly I:C treatment.

(E) Schematic of inducible knockdown of IGF2BP3 in MLL-Af4 leukemia model using MLL-Af4 transduced mouse HSPCs from IMP3f/f;Mx1-Cre mice.

(F, G) Loss of *Igf2bp3* expression prior to poly I:C induction in MLL-Af4 transduced mouse HPSCs from *Igf2bp3$^{f/f}$;Mx1-Cre* mice ("I3CKO") by RT-qPCR and by Western blot analysis.

(H) Decreased cell growth in I3CKO cells versus WT.

(I) Deceased total colony number in methylcellulose colony formation assays in I3CKO cells versus WT.

Supplemental Tables

Supplemental Table S1. Single-guide RNA sequences and qPCR primer sequences

sgRNA sequences		
Name	**Target**	**Sequence**
NT	Non-targeting (human)	TAGACAACCGCGGAGAATGC
I3-sg2	*IGF2BP3* (human), *Igf2bp3* (mouse)	ATTCCAGTAAGGACCAAGCT
NT	Non-targeting (mouse)	GAGGTATTCGGCTCCGCG
NT-2	Non-targeting (mouse)	ATGTTGCAGTTCGGCTCGAT
I3-sg3	*Igf2bp3* (mouse)	GAATCCATTCAGTTTGTCTA
Mouse qPCR primers		
Gene	**Forward primer**	**Reverse primer**
Fcnb	CACTATTCGTCTTGACCCTGAC	GGTCCAGTTGGTCCCTCTTT
Prg2	TGAAACTTCTGACTCCAAAAGCC	CGGCATTAGCTCTTCCCCT
Mmrn1	GGTCTTCAGGCTTACCAACAC	GAGTGGCCGAGAGCACTTG
Ets1	GGGTGATGTGGGCTGTGAAT	TGGGTAGGTAGGGTTGGCTC
Cebpe	GCAGCCACTTGAGTTCTCAGG	GATGTAGGCGGAGAGGTCGAT
Cebpd	CGACTTCAGCGCCTACATTGA	CTAGCGACAGACCCCACAC
Elane	AGCAGTCCATTGTGTGAACGG	CACAGCCTCCTCGGATGAAG
Pram1	GAAACCTTCATATCCTCAAGCCA	GCTGTGGATGCTTCTTAGGGAA
Igf2bp3	CCTGGTGAAGACGGGCTAC	TCAACTTCCATCGGTTTCCCA
Hoxa9	AAAACACCAGACGCTGGAAC	TCT TTTGCTCGGTCCTTGTT
Myc	ATGCCCCTCAACGTGAACTTC	CGCAACATAGGATGGAGAGCA
Spib	AGGAGTCTTCTACGACCTGGA	GAAGGCTTCATAGGGAGCGAT
PML	CAGGCCCTAGAGCTGTCTAAG	ATACACTGGTACAGGGTGTGC
CDK6	TCTCACAGAGTAGTGCATCGT	CGAGGTAAGGGCCATCTGAAAA

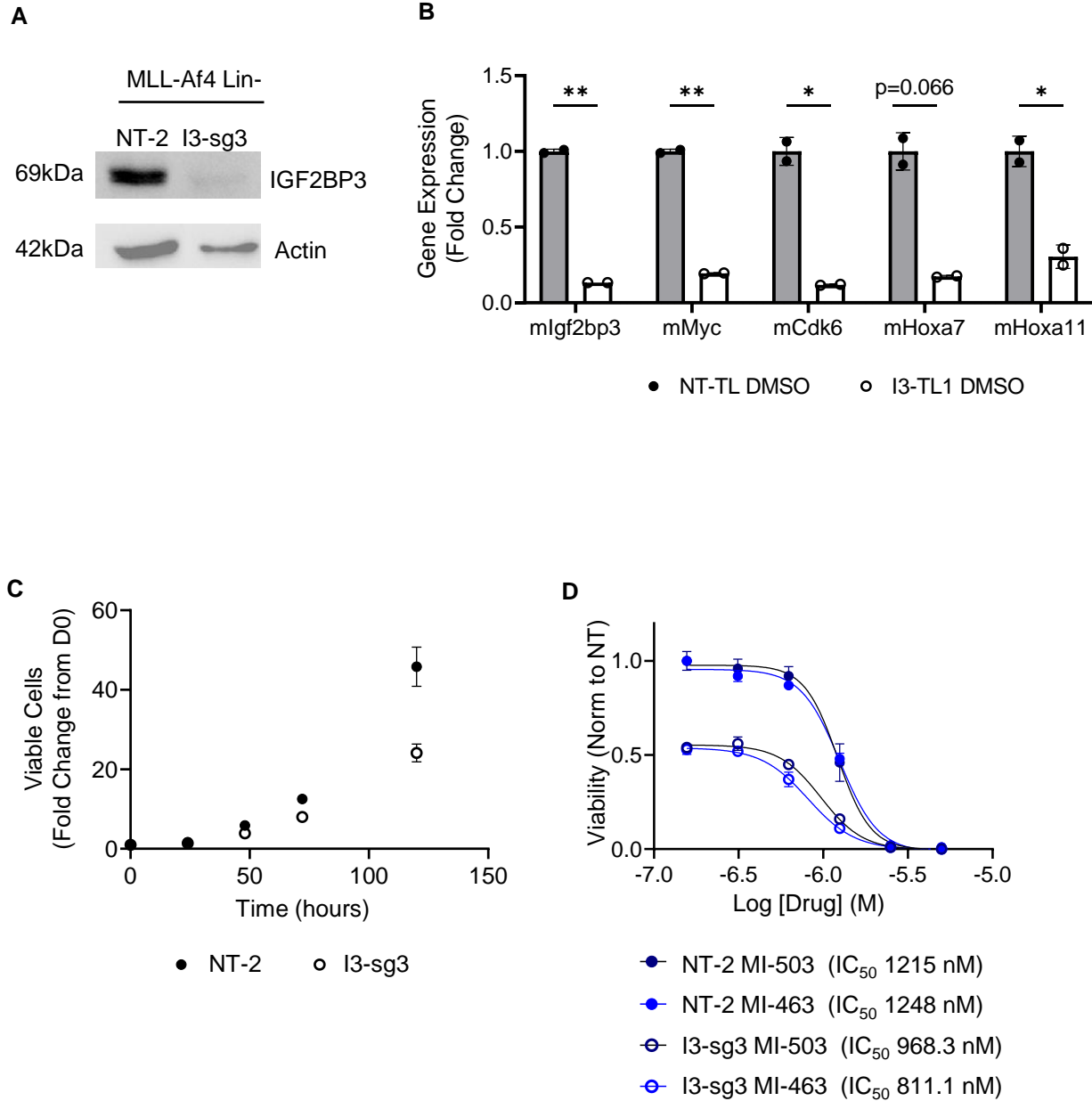|		
L32	AAGCGAAACTGGCGGAAAC	TAACCGATGTTGGGCATCAG
Hoxa10	GAAGAAACGCTGCCCTTACA	GATTCGGTTTTCTCGGTTCA
Hoxa7	ATGTGAACGCGCTTTTTAGC	ATTGTATAAGCCCGGCACAG
Meis1	CTCCCTTCAGTGCAGCAGTT	CTGTCAATCACAGGCGAGGT

Supplemental Table S2. Flow antibodies

Antibody	Vendor/Catalog Number
CD45.1 APC/Cy7	Biolegend, 110716
CD45.2 APC	Biolegend 109814
CD11b PE/Cy7	Biolegend, 101216
B220 PerCP/Cy5.5	Biolegend, 103236
Annexin V BV421	BD Biosciences, B563873
Ki67 PerCP/Cy5.5	Biolegend, 652423
CD3e-Biotin	Biolegend 100304
CD4-Biotin	eBioscience 13-0041-82
CD8-Biotin	Biolegend 100704
B220-Biotin	Biolegend 103204
NK1.1-Biotin	Biolegend 108703
Ter119-Biotin	Biolegend 116204
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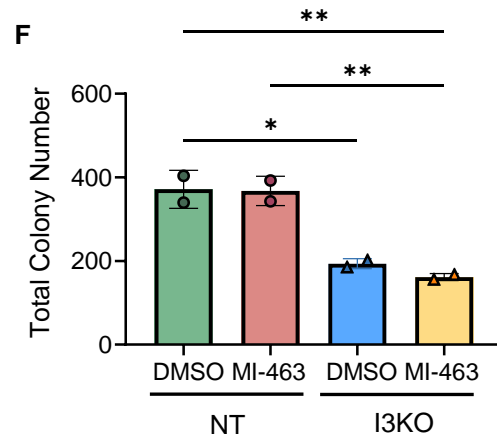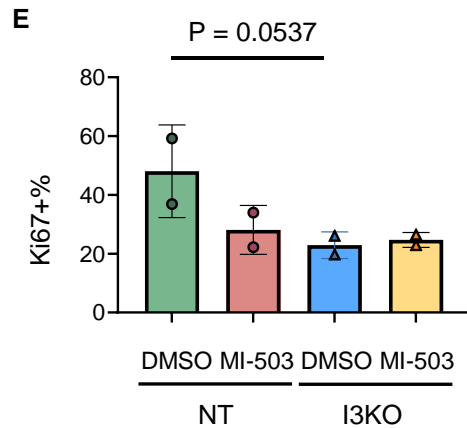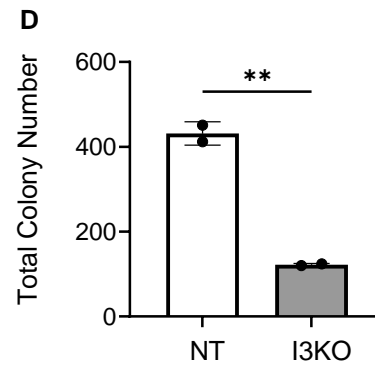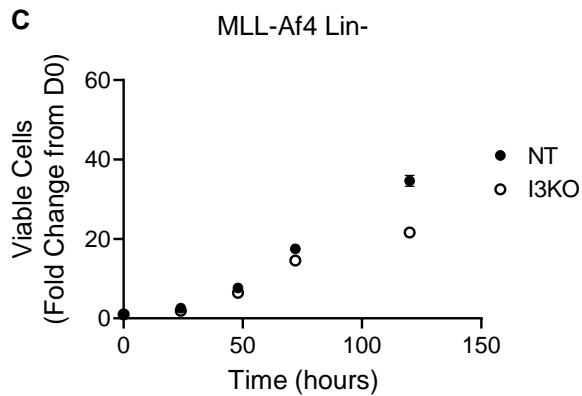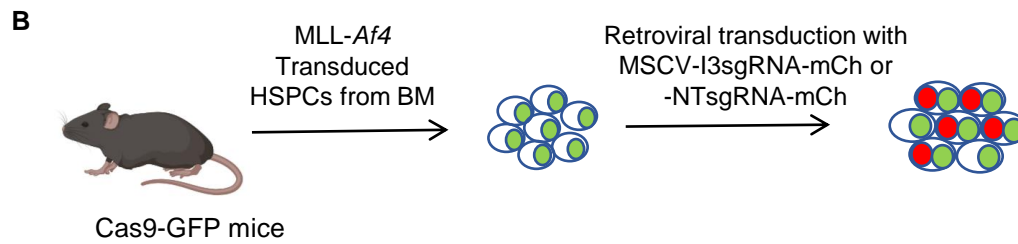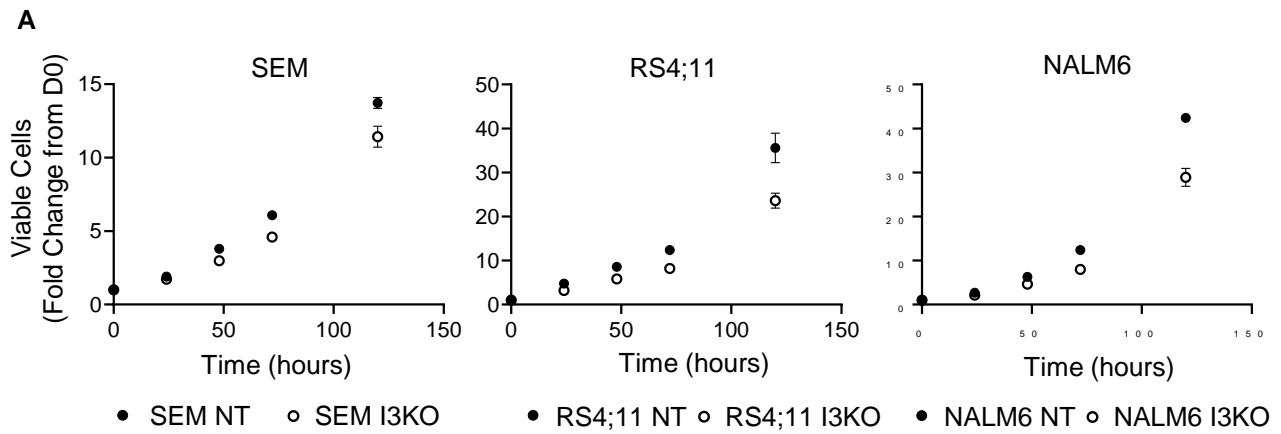
Figure S1. IGF2BP3 knockdown in human B-ALL cell lines and murine MLL-Af4 Lin- HSPCs decreases cell growth.

A

MLL-Af4 Lin-

NT-2   I3-sg3

69kDa   IGF2BP3

42kDa   Actin

B

C

- NT-2   ○ I3-sg3

D

- NT-2 MI-503 (IC$_{50}$ 1215 nM)
- NT-2 MI-463 (IC$_{50}$ 1248 nM)
- ○ I3-sg3 MI-503 (IC$_{50}$ 968.3 nM)
- ○ I3-sg3 MI-463 (IC$_{50}$ 811.1 nM)

Figure S2. IGF2BP3 depletion, using alternative guides targeting IGF2BP3, show expected changes in downregulation of known IGF2BP3 targets, decreased cell growth, and sensitization to menin-MLL inhibition.

A

NT DMSO

B

NT MI-503

C

KO DMSO

D

KO MI-503
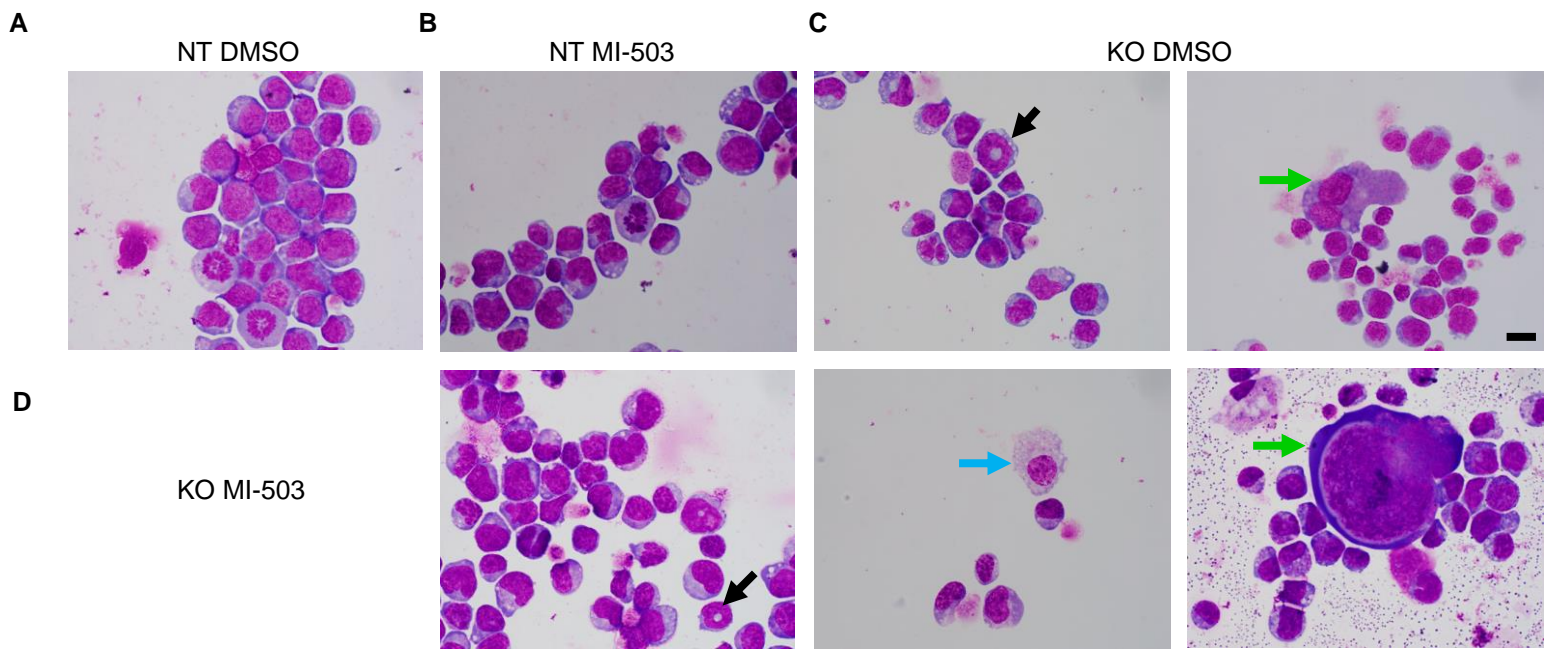
Figure S3. Increased differentiation in MLL-Af4 leukemic cells seen with I3KO and MI-503 by morphology.

A

	Comparison	↑	↓	Total
I3KO vs NT	I3KO vs. NT DMSO	4346	1720	6066
MI-503 vs DMSO	NT 0.2 μM vs. DMSO	5	6	11
	NT 1.0 μM vs. DMSO	245	495	740
	I3KO 0.2 μM vs. DMSO	18	260	278
	I3KO 1.0 μM vs. DMSO	390	307	697

B NT vs I3KO
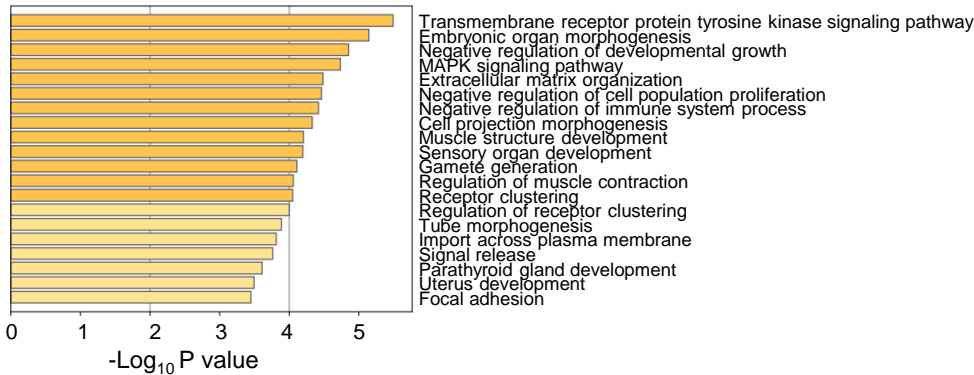
C NT, vehicle vs MI-503
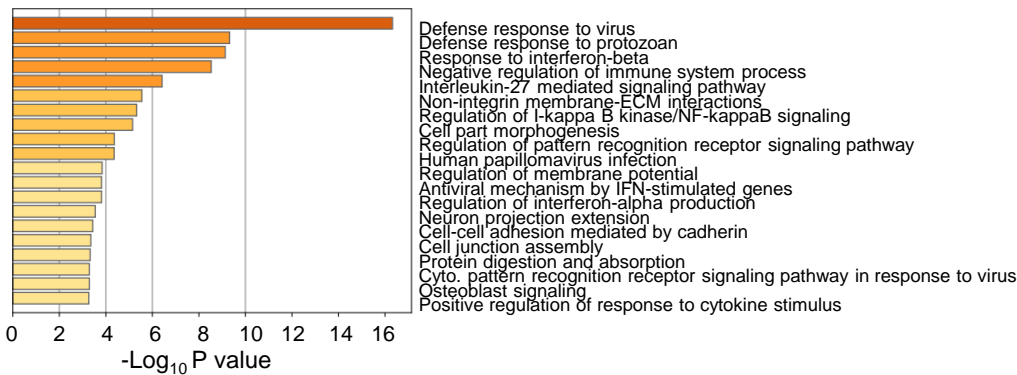
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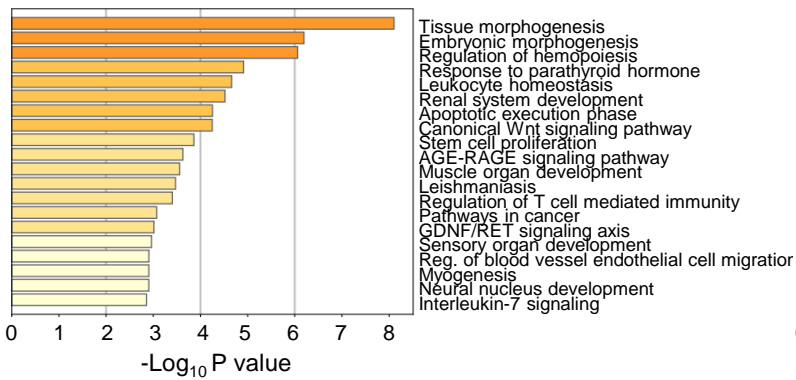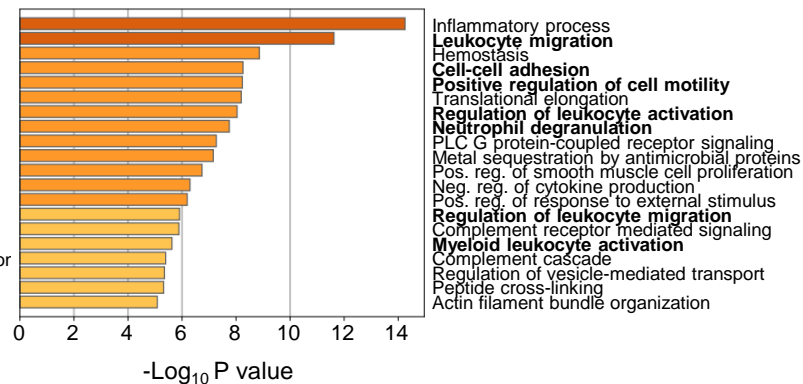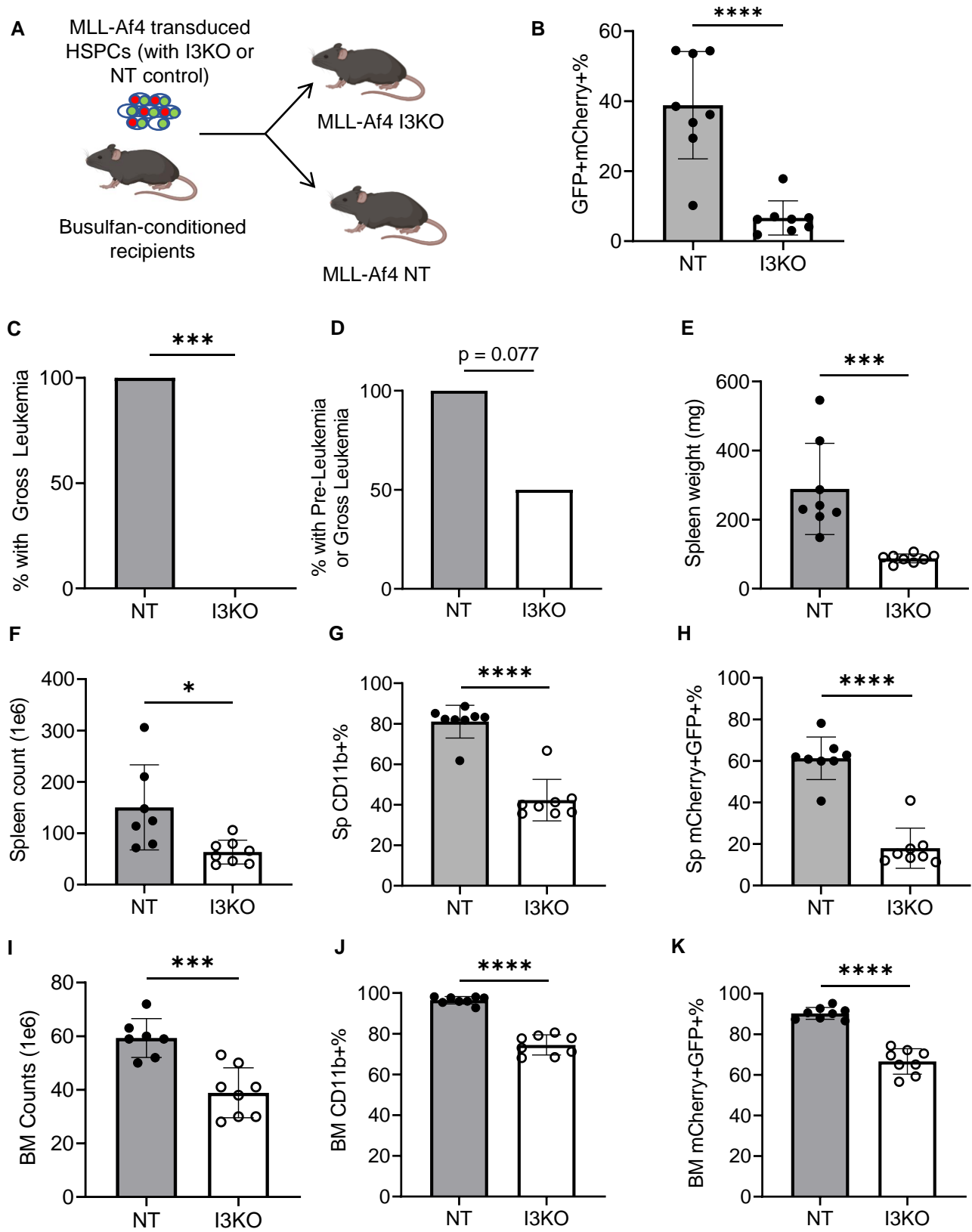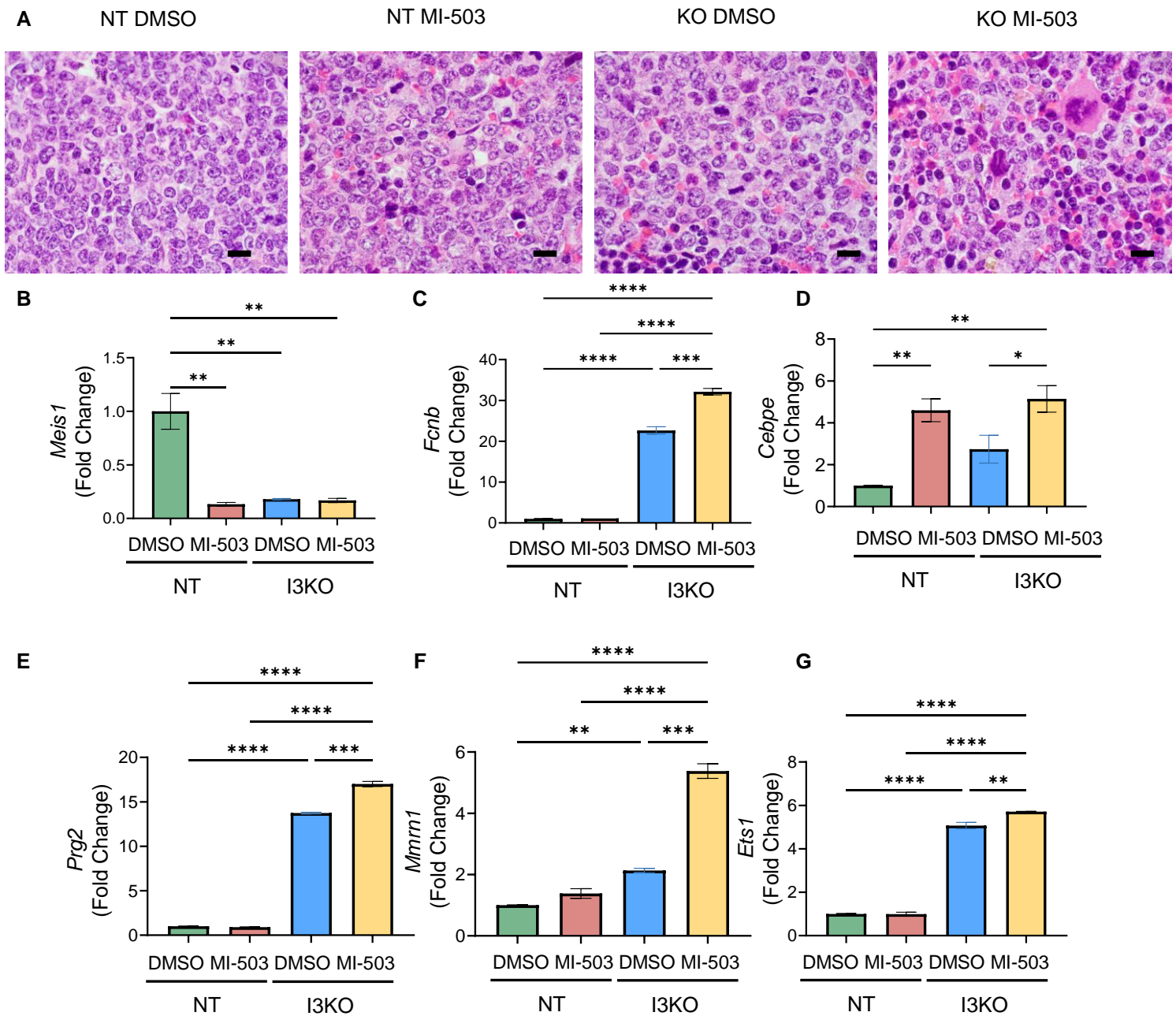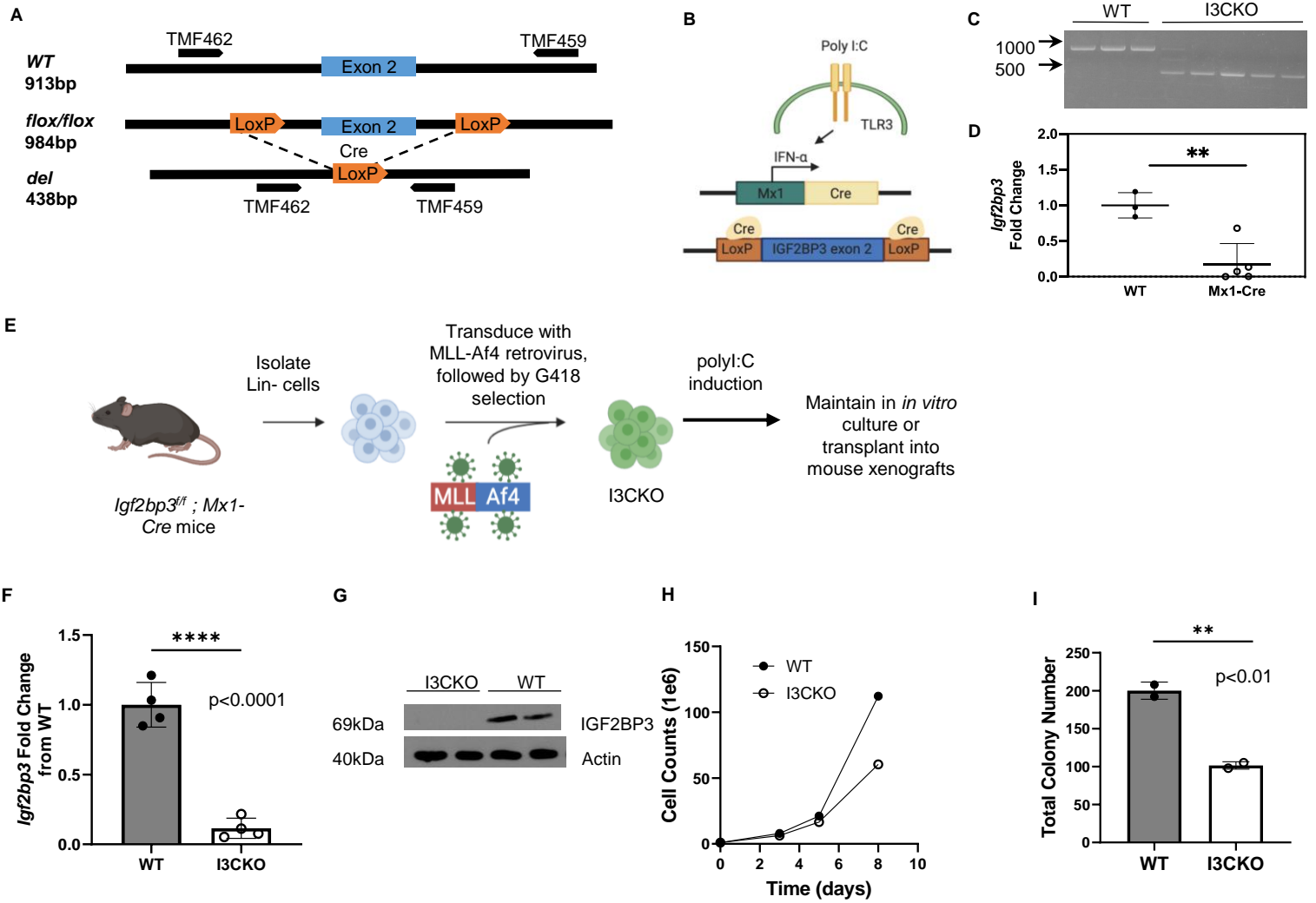

Figure S4. Differentially expressed genes in MLL-Af4 Lin- cells with depletion of IGF2BP3 and treatment with menin-MLL inhibitor, MI-503.

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Figure S6. Additional histology and validation of individual RT-qPCR genes upregulated in differentiation pathways in mice transplanted with MLL-Af4 leukemia cells, depleted for IGF2BP3 and treated with MI-503 *in vitro*

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