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## Supplemental Information

# Proto-Oncogenic Role of Mutant *IDH2* in Leukemia Initiation and Maintenance

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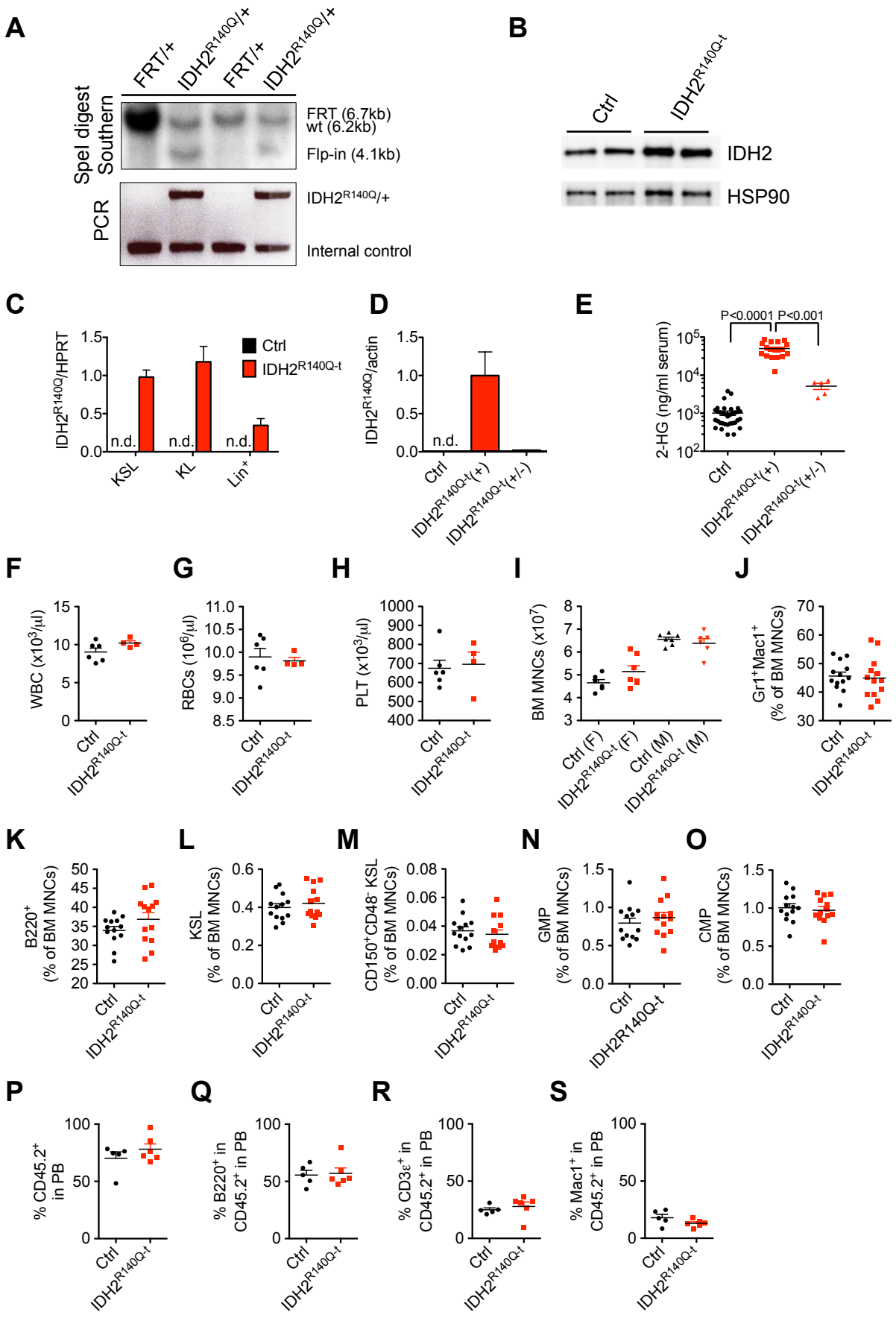
A
FRT/+
IDH2R140Q/+
FRT/+
IDH2R140Q/+
SpeI digest
Southern
FRT (6.7kb)
wt (6.2kb)
Flp-in (4.1kb)
PCR
IDH2R140Q/+
Internal control
B
Ctrl
IDH2R140Q-t
IDH2
HSP90
C
IDH2R140Q/HPRT
Ctrl
IDH2R140Q-t
n.d.
KSL
KL
Lin+
D
IDH2R140Q/actin
n.d.
Ctrl
IDH2R140Q-t(+)
IDH2R140Q-t(+/-)
E
P<0.0001
P<0.001
2-HG (ng/ml serum)
Ctrl
IDH2R140Q-t(+)
IDH2R140Q-t(+/-)
F
WBC (x10³/μl)
Ctrl
IDH2R140Q-t
G
RBCs (10⁶/μl)
Ctrl
IDH2R140Q-t
H
PLT (x10³/μl)
Ctrl
IDH2R140Q-t
I
BM MNCs (x10⁷)
Ctrl (F)
IDH2R140Q-t (F)
Ctrl (M)
IDH2R140Q-t (M)
J
Gr1+Mac1+ (% of BM MNCs)
Ctrl
IDH2R140Q-t
K
B220+ (% of BM MNCs)
Ctrl
IDH2R140Q-t
L
KSL (% of BM MNCs)
Ctrl
IDH2R140Q-t
M
CD150+CD48- KSL (% of BM MNCs)
Ctrl
IDH2R140Q-t
N
GMP (% of BM MNCs)
Ctrl
IDH2R140Q-t
O
CMP (% of BM MNCs)
Ctrl
IDH2R140Q-t
P
% CD45.2+ in PB
Ctrl
IDH2R140Q-t
Q
% B220+ in CD45.2+ in PB
Ctrl
IDH2R140Q-t
R
% CD3ε+ in CD45.2+ in PB
Ctrl
IDH2R140Q-t
S
% Mac1+ in CD45.2+ in PB
Ctrl
IDH2R140Q-t

**Figure S1, related to Figure 1. Generation and Characterization of *IDH2*$^{R140Q\text{-}t}$ Mice.**

(A) Southern blot (upper panel) and PCR on genomic DNA confirming that integration had occurred as expected.

(B) Western blot of BM MNCs from two Ctrl and two *IDH2*$^{R140Q\text{-}t}$ mice following 8 weeks of transgene induction. The total amount of IDH2 protein (i.e. endogenous wild-type IDH2 and transgene IDH2$^{R140Q}$) in *IDH2*$^{R140Q\text{-}t}$ mice is approximately ~2 fold higher than in littermate controls.

(C) qRT-PCR using primers specific for the transgene of hematopoietic stem/progenitor cells (KSL; cKit$^{+}$Sca1$^{+}$Lin$^{-}$), myeloid progenitors (KL; cKit$^{+}$Sca1$^{-}$ Lin$^{-}$) or mature (Lin$^{+}$) cells sorted from control (Ctrl) or *IDH2*$^{R140Q\text{-}t}$ mice following 8 weeks of transgene induction. Error bars, mean ± SEM (n = 3 mice). n.d., not detected.

(D) qRT-PCR analysis using primers specific for the transgene on peripheral blood of Ctrl and *IDH2*$^{R140Q\text{-}t}$ mice. Blood was collected following 8 weeks of transgene induction (+), and again from the same mice following 2 weeks of transgene de-induction (+/-). Error bars, mean ± SEM (n = 2 mice/group). n.d., not detected.

(E) Serum levels of 2-HG measured by LC-MS. Serum was collected following 8 weeks of transgene induction (+) or 8 weeks of induction followed by 2 weeks of de-induction (+/-).

(F-H) Automated cell counts in peripheral blood.

(I) Total number of bone marrow mononuclear cells recovered from the femurs and tibias of control and *IDH2*$^{R140Q\text{-}t}$ animals.

(J-O) Percentage of various differentiated, progenitor and stem cell populations in the bone marrow or spleen of control and *IDH2*$^{R140Q\text{-}t}$ animals. Error bars, mean ± SEM.

(P) Percentage of CD45.2$^{+}$ donor cells in the peripheral blood of CD45.1$^{+}$ recipients transplanted with KSL cells from control or *IDH2*$^{R140Q\text{-}t}$ animals at 16 weeks post transplant. Error bars, mean ± SEM.

(Q-S) Percentage of B, T and myeloid cells within the CD45.2$^{+}$ donor cell fraction in the peripheral blood of CD45.1$^{+}$ recipients transplanted with KSL cells from control or *IDH2*$^{R140Q\text{-}t}$ animals at 16 weeks post transplant. Error bars, mean ± SEM.

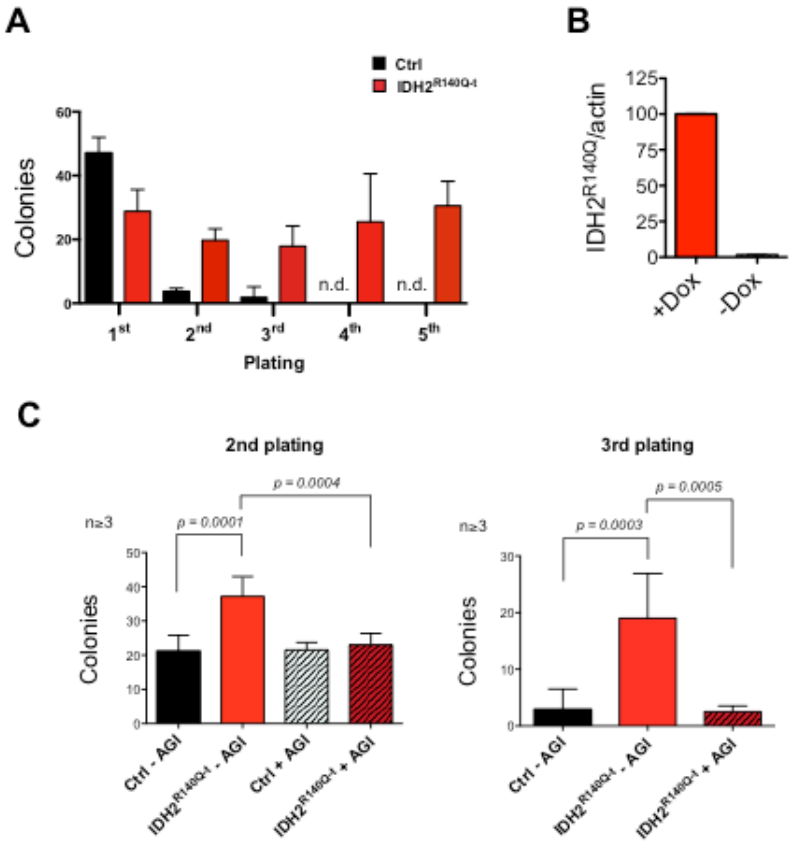


**Figure S2, related to Figures 2. *IDH2*$^{R140Q-t}$ Induces a Block of Erythroid Differentiation in KSL Cells**

(A) Methylcellulose CFU-GEMM assay using KSL cells sorted from *IDH2*$^{R140Q-t}$ or control mice following 8 weeks of doxycycline treatment. Doxycycline was added to the media to maintain transgene expression throughout the differentiation process. For the serial re-plating experiment, cells were re-plated as indicated in the methods.

(B) qRT-PCT analysis on BM MNCs isolated from *IDH2*$^{R140Q-t}$ mice following 8 weeks of doxycycline and cultured with or without doxycycline.

(C) AGI-6780 reverses the aberrant re-plating activity of *IDH2*$^{R140Q-t}$ cells in the second and third plating.

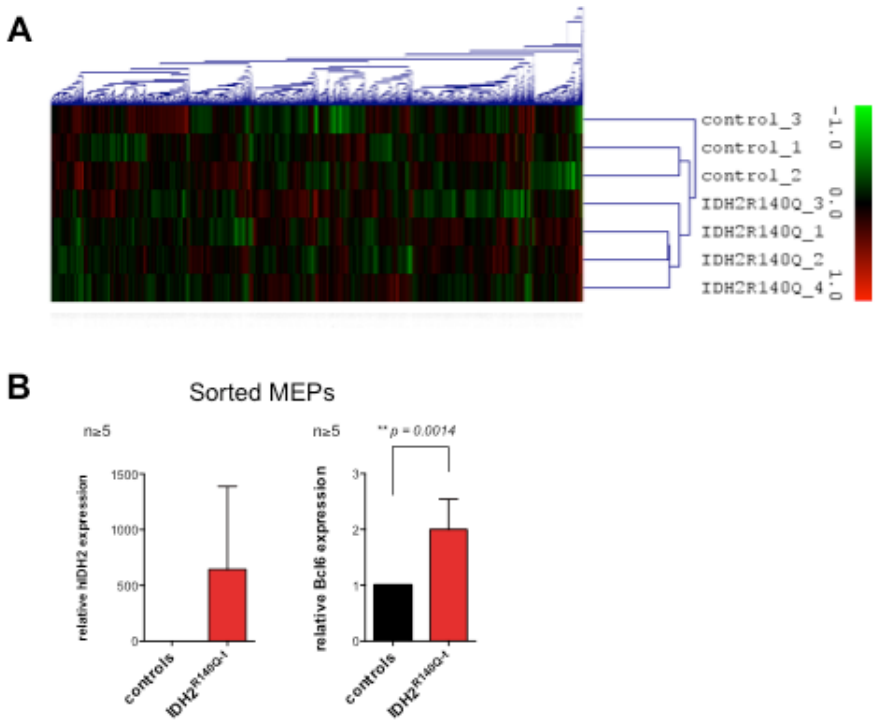


**Figure S3, related to Figure 3. *IDH2*$^{R140Q\text{-}t}$ Induces Transcriptional Alterations in Hematopoietic Cells Undergoing Differentiation**

(A) Unsupervised Hierarchical Clustering of CFU-GEMM Samples from *IDH2*$^{R140Q\text{-}t}$ or Control Mice. All analyzed transcripts are shown.

(B) *IDH2*$^{R140Q}$ is expressed and *Bcl-6* mRNA is upregulated in megakaryocyte erythroid progenitors (MEPs) sorted from *IDH2*$^{R140Q\text{-}t}$ mice. Error bars, mean ± SEM, n ≥ 5 mice/group.

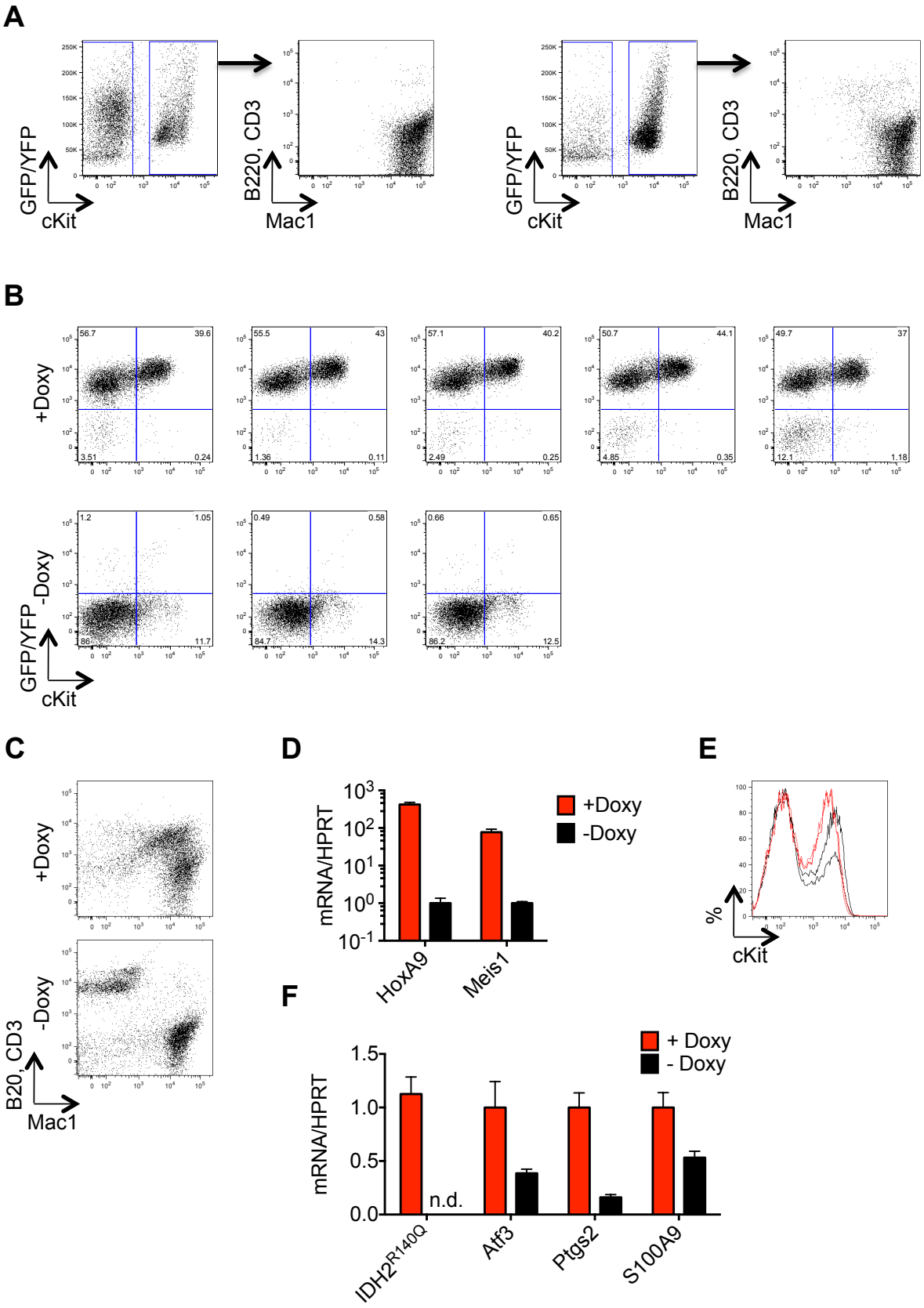
A
GFP/YFP
cKit
B220, CD3
Mac1
GFP/YFP
cKit
B220, CD3
Mac1
B
+Doxy
-Doxy
GFP/YFP
cKit
C
+Doxy
-Doxy
B20, CD3
Mac1
D
mRNA/HPRT
+Doxy
-Doxy
HoxA9
Meis1
E
%
cKit
F
mRNA/HPRT
+ Doxy
- Doxy
n.d.
IDH2R140Q
Atf3
Ptgs2
S100A9

**Figure S4, related to Figure 4. Continued Expression of *IDH2*$^{R140Q}$ is Required for Proliferation and/or Maintenance of HoxA9/Meis1a-Transformed Leukemic Cells**

(A) FACS plots of bone marrow from individual primary recipients showing the characteristic presence of GFP/YFP$^{+}$Mac1$^{+}$ cells 7 weeks after being transplanted with HoxA9/Meis1a transduced *IDH2*$^{R140Qhm\text{-}t}$ KSL cells. GFP/YFP$^{+}$ were sorted and used in secondary transplantation experiments.

(B) FACS plots of bone marrow from individual secondary recipients untreated or treated with doxycycline. Disease onset and development in doxycycline treated recipients is almost identical.

(C) Representative FACS plots of bone marrow from secondary recipients untreated or treated with doxycycline. Untreated recipients displayed normal levels of myeloid and lymphoid cells.

(D) qRT-PCR for *HoxA9* and *Meis1a* confirming overexpression. Error bars, mean ± SEM (n = 3 mice/group).

(E) FACS plots of BM MNCs from 2 doxycycline treated (red lines) and 2 untreated (black lines) recipients at the 4-week time point showing distribution of the stem/progenitor marker cKit.

(F) qRT-PCR on GFP/YFP$^{+}$ cells sorted from the bone marrow of transplanted recipients at the 4 weeks time point (2 weeks off doxycycline). Error bars, mean ± SEM (n = 2 mice/group).

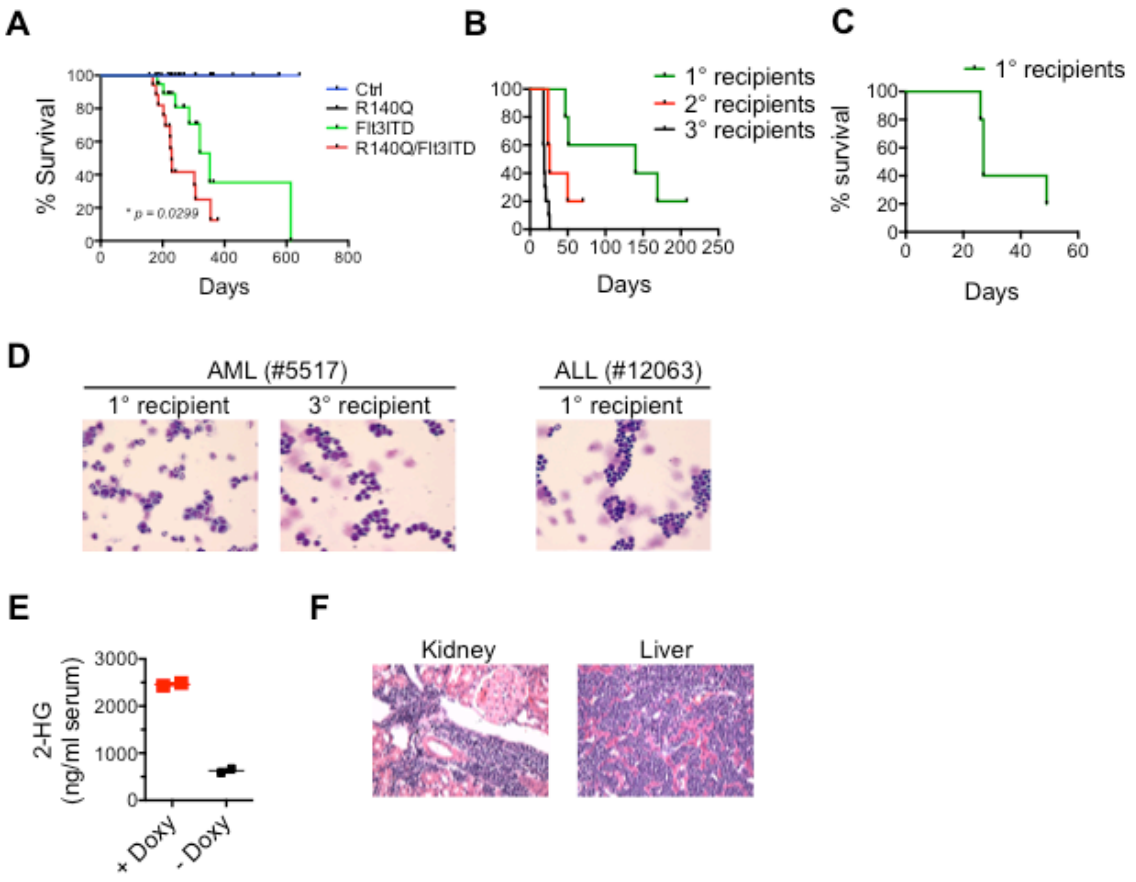


**Figure S5, related to Figures 5 and 6. Mutant IDH2 Contributes to Leukemia Initiation and Maintenance in the Context of a Co-operating *Flt3$^{ITD}$* Lesion.**

(A) Kaplan-Meier survival curves for *IDH2$^{R140Q-t}$;Flt3$^{ITD}$* (n=17), *Flt3$^{ITD}$* mice (n=21), *IDH2$^{R140Q-t}$* (n=8) and control mice (n=23). Median survival of *IDH2$^{R140Q-t}$;Flt3$^{ITD}$* animals was 229 days, versus 352 days for *Flt3$^{ITD}$* mice (p=0.0299, Mantel-Cox test).

(B) Kaplan-Meier survival curves for primary, secondary and tertiary recipients transplanted with leukemic cells from recipient #5517 diagnosed with AML-like disease. The recipients were treated with doxycycline (n ≥ 5 recipients/transplantation).

(C) Kaplan-Meier survival curves for primary recipients transplanted with leukemic cells from recipient #12063 diagnosed with ALL-like disease. The recipients were treated with doxycycline (n = 5 recipients/transplantation).

(D) May-Grunwald-Giemsa-stained bone marrow cytospins showing morphology of leukemic blasts in doxycycline treated recipients transplanted with AML-like or ALL-like leukemic cells.

(E) Serum 2-HG levels in recipients treated or not with doxycycline. Doxycycline-treated animals display 2-HG levels that are comparable to human AML patients with an IDH mutation. Untreated animals display baseline 2-HG levels comparable to wild-type mice (see **Figure S1E**)

(F) Infiltration of leukemic blasts into non-hematopoietic organs in recipients not treated with doxycycline.

**Table S1, related to Figure 2. Differentially Expressed Transcripts in KSL Cells.**

KSL cells were sorted from control and *IDH2*$^{R140Q\text{-}t}$ mice and cultured for 3 days in the methylcellulose CFU-GEMM assay (P value < 0.05, fold change >1.5). See separate file.

**Table S2, related to Figure 2. Gene Set Enrichment Analysis of Microarray Data - Biological Pathways Down-Regulated in *IDH2*$^{R140Q\text{-}t}$ Cells Compared with Controls.**

| gene set from MSigDB | SIZE | ES | NES | NOM p-val | FDR q-val | details of the gene set |
|---|---|---|---|---|---|---|
| REACTOME_SYNTHESIS_OF_DNA | 80 | -0.5463 | -2.1983 | 0 | 0.0038 | Genes involved in Synthesis of DNA |
| MANALO_HYPOXIA_DN | 245 | -0.4626 | -2.1864 | 0 | 0.0025 | Genes down-regulated in response to both hypoxia and overexpression of an active form of HIF1A |
| REACTOME_ACTIVATION_OF_ATR_IN_RESPONSE_TO_REPLICATION_STRESS | 35 | -0.6491 | -2.1785 | 0 | 0.0021 | Genes involved in Activation of ATR in response to replication stress |
| REACTOME_DNA_REPAIR | 80 | -0.4893 | -1.9867 | 0 | 0.0134 | Genes involved in DNA Repair |
| KEGG_DNA_REPLICATION | 33 | -0.5918 | -1.9854 | 0 | 0.0126 | DNA replication |
| IVANOVA_HEMATOPOIESIS_EARLY_PROGENITOR | 361 | -0.3799 | -1.855 | 0 | 0.0417 | Genes in the expression cluster 'Early Progenitors Shared': up-regulated in hematopoietic progenitors from adult bone marrow and from fetal liver. |
| KEGG_MISMATCH_REPAIR | 20 | -0.6252 | -1.7883 | 0.0039 | 0.0624 | KEGG_MISMATCH_REPAIR |
| WELCH_GATA1_TARGETS | 16 | -0.5861 | -1.5817 | 0.0281 | 0.1469 | Genes up-regulated after GATA1 activation in G1E-ER4 cells (erythroid precursors engineered to express GATA1 upon addition of estradiol ). |
| FUJII_YBX1_TARGETS_DN | 165 | -0.3482 | -1.5594 | 0.002 | 0.166 | Genes down-regulated in MCF-7 cells (breast cancer) after knockdown of YBX1 by RNAi. |
| YU_BAP1_TARGETS | 27 | -0.4887 | -1.5363 | 0.0247 | 0.182 | Genes deregulated in U2OS cells (osteosarcoma) upon knockdown of BAP1 by RNAi. |
| HUANG_GATA2_TARGETS_DN | 57 | -0.4016 | -1.5011 | 0.0225 | 0.1981 | Genes down-regulated in G1ME cells (megakaryocyte/erythroid progenitor lacking GATA1 upon knockdown of GATA2 by RNAi. |

## Table S3, related to Figure 2. Gene Set Enrichment Analysis of Microarray Data - Biological Pathways Up-Regulated in *IDH2*$^{R140Q\text{-}t}$ Cells Compared with Controls.

| gene set from MSigDB | SIZE | ES | NES | NOM p-val | FDR q-val | details of the gene set |
|---|---|---|---|---|---|---|
| BROWN_MYELOID_CELL_DEVELOPMENT_UP | 111 | 0.7226 | 3.0406 | 0 | 0 | Genes defining differentiation potential of the bipotential myeloid cell line FDB |
| TIAN_TNF_SIGNALING_VIA_NFKB | 22 | 0.8178 | 2.5312 | 0 | 0 | Genes modulated in HeLa cells by TNF via NFKB pathway |
| PHONG_TNF_TARGETS_UP | 43 | 0.7006 | 2.4842 | 0 | 0 | Genes up-regulated in Calu-6 cells at 1 h time point after TNF treatment. |
| SEKI_INFLAMMATORY_RESPONSE_LPS_UP | 52 | 0.642 | 2.3683 | 0 | 7.37E-05 | Genes up-regulated in hepatic stellar cells after stimulation with bacterial lipopolysacharide (LPS). |
| RASHI_NFKB1_TARGETS | 16 | 0.8247 | 2.3383 | 0 | 6.37E-05 | Known and putative targets of NFKB1 identified among the ATM dependent, late responders to ionizing radiation. |
| KAMIKUBO_MYELOID_CEBPA_NETWORK | 28 | 0.7112 | 2.2751 | 0 | 1.69E-04 | Network of differentially expressed myeloid genes centered around CEBPA. |
| GERY_CEBP_TARGETS | 83 | 0.5619 | 2.2646 | 0 | 1.55E-04 | Genes changed in NIH 3T3 cells by expression of one or more of C/EBP proteins. |
| PID_IL6_7PATHWAY | 37 | 0.6548 | 2.2473 | 0 | 1.43E-04 | IL6-mediated signaling events |
| KEGG_TOLL_LIKE_RECEPTOR_SIGNALING_PATHWAY | 68 | 0.5612 | 2.1714 | 0 | 4.03E-04 | Toll-like receptor signaling pathway |
| TAVOR_CEBPA_TARGETS_UP | 36 | 0.6307 | 2.1366 | 0 | 7.33E-04 | Genes up-regulated in KCL22 cells (CML) by expression of CEBPA |
| VERHAAK_AML_WITH_NPM1_MUTATED_UP | 114 | 0.5007 | 2.1217 | 0 | 8.09E-04 | Genes up-regulated in acute myeloid leukemia (AML) patients with mutated NPM1. |
| REACTOME_INTERFERON_GAMMA_SIGNALING | 38 | 0.6073 | 2.1086 | 0 | 0.001 | REACTOME_INTERFERON_GAMMA_SIGNALING |
| KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY | 41 | 0.5855 | 2.0967 | 0 | 0.0011 | KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY |
| VILIMAS_NOTCH1_TARGETS_DN | 15 | 0.7839 | 2.0917 | 0 | 0.0012 | Genes down-regulated in bone marrow progenitors by constitutively active NOTCH1. |
| DAUER_STAT3_TARGETS_UP | 36 | 0.6204 | 2.0851 | 0 | 0.0013 | Top 50 genes up-regulated in A549 cells expressing STAT3 off an adenovirus vector. |
| DELPUECH_FOXO3_TARGETS_UP | 50 | 0.5713 | 2.0828 | 0 | 0.0013 | Genes up-regulated in DL23 cells upon expression of an activated form of FOXO3. |
| HUANG_GATA2_TARGETS_UP | 124 | 0.482 | 2.0718 | 0 | 0.0015 | Genes up-regulated in G1ME cells (megakaryocyte/erythroid progenitor lacking GATA1) upon knockdown of GATA2. |
| PID_PDGFRBPATHWAY | 113 | 0.4685 | 2.0017 | 0 | 0.0032 | PDGFR-beta signaling pathway |
| WINTER_HYPOXIA_METAGENE | 186 | 0.4349 | 1.9716 | 0 | 0.0045 | Genes regulated by hypoxia, based on literature searches. |
| HOUSTIS_ROS | 26 | 0.6064 | 1.9352 | 0.00214 | 0.0065 | Genes known to modulate ROS or whose expression changes in response to ROS. |
| CHEN_HOXA5_TARGETS_9HR_UP | 149 | 0.3569 | 1.5585 | 0 | 0.0687 | Genes up-regulated 9 h after induction of HoxA5 expression in a breast cancer cell line. |

**Table S4. Oligonucleotide Primers Used for qRT-PCR Analysis.**

| | |
|---|---|
| mNdrg2_fwd | GCAAGCTGACAGAGGCTTTC |
| mNdrg2_rev | GATGCTGCACTGGTCAGAGA |
| mThy1_fwd | TCCTGCTCTCAGTCTTGCAG |
| mThy1_rev | TATTCTCATGGCGGCAGTCC |
| mIl7r_fwd | GAAAGCAACTGGACGCATGT |
| mIl7r_rev | TGGGGAATGGATCGGACTTTG |
| mCcr1_fwd | CCATCATCATACAGGAAGCCAAG |
| mCcr1_rev | ACAGTGAGTCTGTGTTTCCAGAG |
| mBcl6_fwd | GAGTCGGGACATCTTGACGG |
| mBcl6_rev | TACTGTAGAACAGGCCGCTG |
| mMs4a7_fwd | GCTGGAGGTTTTCCTAGCTG |
| mMs4a7_rev | TGATCTTGTGATTGAGGCAAG |
| hIDH2_fwd | GCAGAGCCTCAGGCTCGCG |
| hIDH2_rev | GGCTGCTCTTGCGAGGT |
| mHprt_fwd | GGTGGAGATGATCTCTCAACTT |
| mHprt_rev | CCAGCAAGCTTGCAACCTTAAC |
| mHoxA9_fwd | TCCTCCAGTTGATAGAGAAAAACA |
| mHoxA9_rev | GCGAGCATGTAGCCAGTTG |
| mMeis1a_fwd | GGGGATAACAGCAGTGAGCA |
| mMeis1a_rev | CCACGCTTTTTGTGACGCTT |
| mPtgs2_fwd | CTGACCCCCAAGGCTCAAAT |
| mPtgs2_rev | TTAAGTCCACTCCATGGCCC |
| mAtf3_fwd | AGGCAGGAGCATCCTTTGTC |
| mAtf3_rev | CTGCTTTGCATAGGACCCCA |
| mS100A9_fwd | AAATGGTGGAAGCACAGTTGG |
| mS100A9_rev | AAGCTCAGCTGATTGTCCTGG |

## SUPPLEMENTAL EXPERIMENTAL PROCEDURES

### Generation of a Tetracycline-inducible *IDH2*$^{R140Q}$ Allele

The targeting vector to generate the tetracycline-inducible *IDH2*$^{R140Q}$ allele is shown in Fig. 1A. The targeting construct was generated by cloning a mutant *IDH2*$^{R140Q}$ cDNA, which was generated by PCR-based mutagenesis, downstream of a tetracycline response element (TRE) followed by a protamine-1 polyA cassette as previously described (Fisher, 2001). The construct was subsequently targeted into the mouse *Collagen A1* locus of C2-embryonic stem (ES) cells using flp recombinase-mediated genomic integration as previously described (Beard et al., 2006). Mouse embryonic stem cells (ES) carrying a single copy of the *IDH2*$^{R140Q}$ construct were initially identified by resistance to the antibiotic marker hygromycin. Hygromycin-resistant ES cell clones were then subjected to Southern blot (Beard et al., 2006) and PCR analysis to confirm correct targeting. Karyotypically normal clones were injected into blastocysts and transplanted into C57BL/6 mice. Founder chimeric mice were then crossed to C57BL/6 and PCR genotyping and Southern blotting confirmed germ-line transmission. Subsequent genotyping of offspring was performed by PCR analysis with primers specific for the *IDH2*$^{R140Q}$: IDH2_sense CTCCCTTGGCCTGATGAC; IDH2_antisense GCCTGCGACGGCGGCATCTGC. The genotyping protocol for the *M2-rtTA* mice can be found at the Jackson Laboratories website (www.jax.org).

**FACS Analysis**

FACS analyses were performed according to standard protocols. In brief, mice were euthanized and single-cell suspensions from the bone marrow (BM) were generated by crushing the tibia and the femur using a mortar and pestle in PBS supplemented with 2% FBS (2%FBS/PBS). For the spleen, single cell suspensions were prepared by mashing the spleen in 2%FBS/PBS. Cell suspensions were passed through 100μM cell strainers, centrifuged and re-suspended in 1-2ml ACK red cell lysis buffer (GIBCO). Red blood cells were lysed on ice for 1min. Cell suspension were then washed in 2%FBS/PBS, centrifuged and re-suspended in 1ml 2%FBS/PBS. Viable cell numbers were determined by cell counting after trypan blue staining. For immunostaining, we used monoclonal antibodies specific for the following: c-Kit (2B8), Sca-1 (E13-161.7), CD3e (145-2C11), B220 (RA3-6B2), Gr-1 (RB6-8C5), CD11b (M1/70),; all were from eBioscience. Anti-CD150 (TC15-12F12.2) and CD48 (HM48-1) antibodies were from BioLegend. For hematopoietic lineage analysis, we used monoclonal antibodies specific for the following: CD3e (145-2C11), B220 (RA3-6B2), Gr-1 (RB6-8C5) and CD11b (M1/70). All antibodies were from eBioscience. For hematopoietic stem/progenitor cell analysis we used monoclonal antibodies specific for the following: c-Kit (2B8), Sca-1 (E13- 161.7), CD150 (TC15-12F12.2) and CD48 (HM48-1). c-Kit and Sca-1 antibodies were from eBioscience and CD150 and CD48 antibodies were from BioLegend. We used a mixture of biotin-labeled monoclonal antibodies against CD4, CD8, CD3e, B220, TER-119, CD11b, Gr-1, IgM, CD19, CD127, and NK-1.1 to deplete Lineage positive cells.

Streptavidin-APCCy7 (E08466-1631) was used as a secondary antibody to detect remaining Lineage positive cells. To assess cell viability, cells were incubated with DAPI prior to FACS analysis. All staining mixtures were analyzed on a BD LSR II flow cytometer (Becton Dickinson). Resulting profiles were further processed and analyzed using the FlowJo 8.7 software.

**FACS Isolation of KSL Cells**

For KSL sorting, bones from hind limbs, hips and spine were collected and crushed in 10-20ml 2%FBS/PBS using a mortar and pestle. After red blood cell lysis in 1-2ml ACK lysis buffer, lineage-negative cells were isolated using a mixture of biotin-labeled monoclonal antibodies against CD4, CD8, CD3e, B220, TER-119, CD11b, Gr-1, IgM, CD19, CD127, and NK-1.1. Next, cells were washed in PBS supplemented with 2mM EDTA (PBS/EDTA) and incubated with anti-streptavidin micro-beads (Miltenyi Biotec) for 15min on ice. After two more washes in PBS/EDTA, lineage depletion was done using a Miltenyi AutoMACS magnetic separator (Miltenyi Biotec). Lineage negative cells were then immunostained with anti-lineage-biotin/streptavidin-APC-Cy7, anti-CD45.2-FITC, anti-cKit-APC and anti-Sca-1-PE antibodies. KSL ($CD45.2^+$ $Lineage^-$ $c\text{-}kit^+$ $Sca1^+$) cells were then sorted on a BD™ FACSAria IIu SORP cell sorter (Becton Dickinson).

**Competitive Transplantation Assay**

KSL cells sorted from *IDH2*$^{R140Q\text{-}t}$ or control donors that had been treated with doxycycline were transplanted into sub-lethally irradiated (6.5 Gr) CD45.1$^{+}$ recipients by retro-orbital injection. 4000 KSL cells/recipient were injected and the recipients were treated with doxycycline. Donor chimerism was assessed using flow cytometry with antibodies specific for CD45.1 or CD45.2, respectively.

**Peripheral Blood Analysis**

For peripheral blood analysis, mice were bled from the submandibular vein. The blood was collected in EDTA-coated tubes and whole blood counts were analyzed on a Hemavet HV950FS counter (Drew Scientific Inc.).

**2-HG Measurement from Mouse Sera**

For the measurement of 2-HG from mouse sera, peripheral blood was collected from the submandibular vein and transferred into EDTA-coated tubes. Serum was isolated by centrifugation at 3000rpm for 15min at 4°C. Extraction of mouse sera was performed by adding 30µL of internal standard to 30µL of mouse serum and 200µL of acetonitrile. Samples were vortexed and subsequently centrifuged at 4°C for 15 min. Supernatants were diluted 1:1 with 100µl deionized water. Samples were then analyzed by liquid chromatography–mass spectrometry as previously described (Dang et al., 2009). Calculations of intracellular mM concentration of 2-HG were based on an average cellular diameter of 16µm.

**Western Blot Analysis**

Cells were lysed in RIPA buffer containing Complete Mini protease inhibitors (EDTA free) (Roche) and a Phosphatase Inhibitor cocktail (Thermo Scientific). 5-50µg of total protein was subjected to SDS–PAGE on 4-12% Bis-Tris acrylamide NuPAGE gels in MOPS SDS running buffer (Invitrogen). The following primary antibodies were used: IDH2 (Novus Biologicals), HSP90 (BD Bioscience; BD Transduction Laboratories), and β-actin (Santa Cruz Biotechnologies). Subsequently, membranes were incubated with secondary, HRP-tagged antibodies (Amersham) and signals were visualized with ECL or ECL plus (Amersham).

**RNA Extraction and Real-Time PCR**

For real-time PCR analyses, RNA was isolated using Trizol (Ambion) with GlycoBlue (Life Technologies) as a carrier. cDNA was synthesized using the High Capacity cDNA Reverse Transcriptase Kit (Applied Biosystems) and analyzed by real-time PCR using the QuantiTect SYBR Green PCR kit (Qiagen) on a Light Cycler 480 System (Roche Applied Science). Manufacturers' recommended protocols were followed throughout. See Supp. Table x for primer sequences.

**Microarray Analysis**

The obtained raw intensity .cel files were normalized by robust multichip analysis (Bioconductor release 2.12) and the quality of cDNA preparation and array hybridization were assessed. Probe sets not expressed or weakly expressed were filtered out and only probe sets expression level above 64 on at least one of the chips were included in the analysis. Differential expression was determined using the limma Bioconductor package by fitting a linear model (Smyth, 2004). Gene set enrichment analysis (Subramanian et al., 2005) was conducted with the gene sets from the Molecular Signatures Database (MolSigDB v3.1). Gene sets with a false discovery rate (FDR) less than 0.1 were selected for further analysis. Genes associated with each significantly regulated pathway were validated and plotted with Cytoscape (Cline et al., 2007) tools with different color intensities reflecting fold change in $IDH2^{R140Q\text{-}t}$ samples relative to controls. The significance of each altered pathway was encoded in the size of the grey rounded squares, with the size of the square being proportional to the absolute value of the associated logarithm transformed FDR.

## SUPPLEMENTAL REFERENCES


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