Supplemental Data

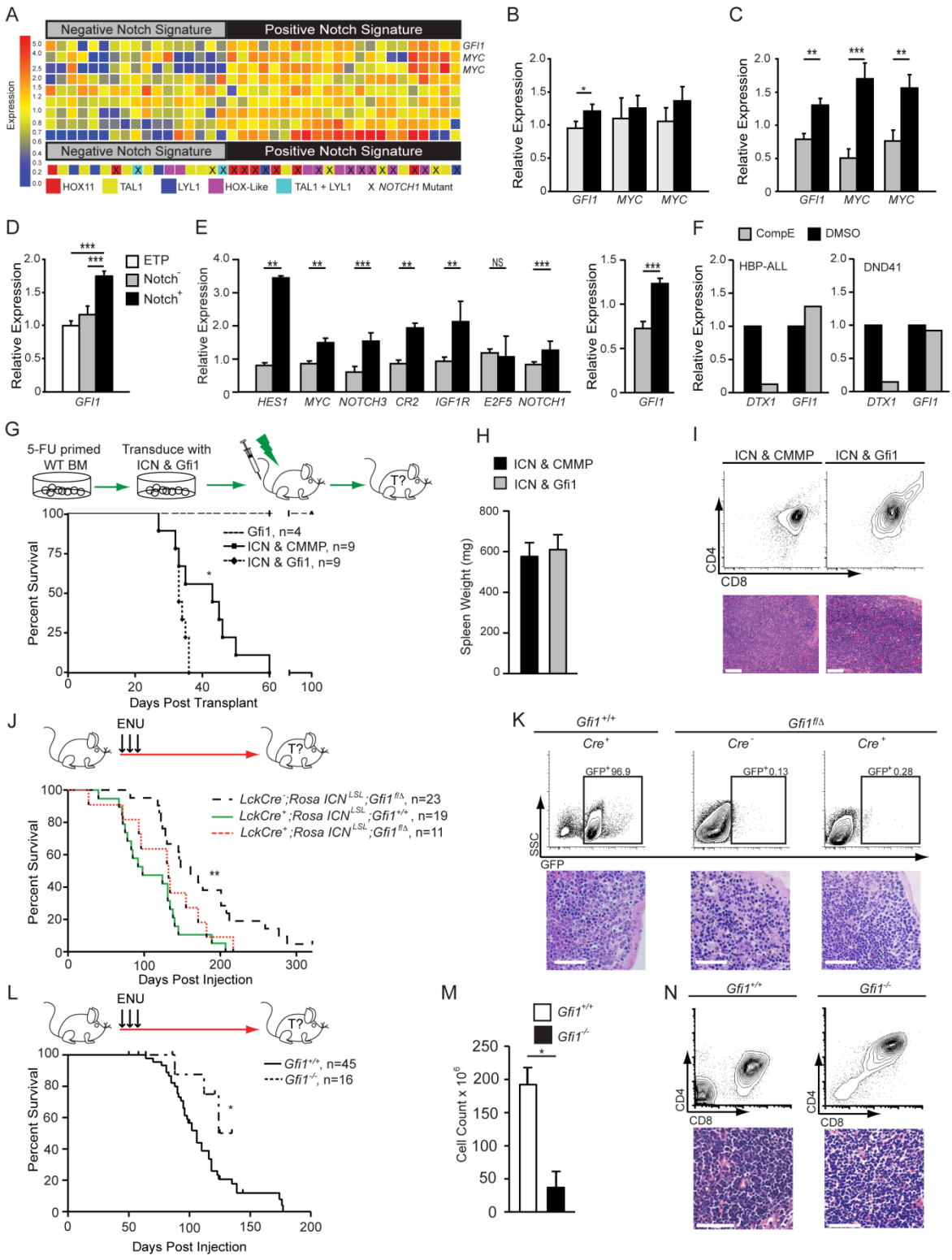

Figure S1, related to Figure 1. *Gfi1* expression is associated with a subgroup of human T-ALL and modulates T-ALL initiation

(A) Expression of published Notch1 target genes was used to classify gene expression array data from 39 T-ALL patients into two groups: "Negative Notch Signature" (grey, left) and "Positive Notch Signature" (black, right).

(B, C) Quantification of mRNA for *GFI1* and two unique probes for the known ICN target gene *MYC* after clustering the patients by *NOTCH1* mutational status (B) or by ICN target gene activation (C).

(D) Expression of published Notch1 target genes or clinical diagnosis was used to classify gene expression array data from 55 T-ALL patients (GSE8879, Figure 1A) into three groups: "ETP-ALL" (white), "Negative Notch Signature" (grey) and "Positive Notch Signature" (black) and relative *GFI1* expression is shown.

(E) Expression of published NOTCH1 target genes was used to classify gene expression array data from another set of 91 T-ALL patients (GSE10609) into two groups "Negative Notch Signature" (grey) and "Positive Notch Signature" (black). (Left) Quantification of mRNA for Notch1 target genes; (right) quantification of mRNA for *GFI1*.

(F) Human T-ALL cell lines were cultured in complete RPMI media with 1 μM Compound E or vehicle control (DMSO) for 16 hours. RNA was isolated and gene expression was analyzed using TaqMan probes for *GFI1* (Hs00382207_m1), *DTX1* (Hs00269995_m1), and normalized to *GAPDH* (Hs00266705_g1) using the ΔΔCT method. One experiment was performed.

(G) Top: Scheme of experimental strategy: 5-FU treated wild-type bone marrow cells were transduced with retroviral vectors expressing GFP and ICN, or GFP alone, then with vectors

expressing DsRed and Gfi1, or DsRed alone, then transplanted into recipient mice. Bottom: Kaplan-Meier curve. ICN and Gfi1 versus ICN versus Gfi1 alone.

(H, I) Post-mortem spleen weight (H) and flow cytometric analysis of thymic tumors (I, top panels) as well as histological sections of splenic tumors (I, bottom panels).

(J) Top: *Lck-Cre*$^{+}$*;RosaICN*^{LSL}(n=19), *RosaICN*^{LSL}*;Gfi1*$^{f/\Delta}$(n=23), and *Lck-Cre*$^{+}$*;RosaICN*^{LSL}*;Gfi1*$^{f/\Delta}$ (n=11) mice were injected with ENU. Cre-mediated deletion of a floxed stop cassette (LSL) activates ICN-IRES-eGFP. Bottom: Kaplan-Meier curve.

(K) Flow cytometric analysis (top panels) and histological sections (bottom panels) from tumors arising in *RosaICN*^{LSL}*;Gfi1*$^{f/\Delta}$, *Lck-Cre*$^{+}$*RosaICN*^{LSL}, and *Lck-Cre*$^{+}$*RosaICN*^{LSL}*Gfi1*$^{f/\Delta}$ mice.

(L) Top: ENU injected *Gfi1*$^{+/+}$ (n=45) and *Gfi1*$^{-/-}$ (n=16) mice were monitored for tumor development and survival. Bottom: Kaplan-Meier curve.

(M, N) Thymic tumor cell numbers (M) and flow cytometric analysis (N, top panels) and histological sections (N, bottom panels) of ENU induced *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ tumors. Scale in histological sections represents 50μm.

| in all Kaplan-Meier curve plots indicate censored mice.

Mean and ±SEM are shown unless stated otherwise. *p<0.05, **p<0.01, ***p<0.001.

Table S1, related to Figures 1. ENU-induced T cell malignancies from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ mice do not harbor mutations in *Notch1* domains associated with T-ALL.

	***Notch1* Domain** (bp sequenced)**:**		
Mouse ENU-treatment	**HD-TAD** (4775-6419)	**HD-PEST** (6321-7708)	**PEST-Stop** (7241-7881)
Gfi1$^{+/+}$ R1	-	-	-
Gfi1$^{+/+}$ R2	-	-	-
Gfi1$^{+/+}$ R3	-	-	-
Gfi1$^{+/+}$ R4	-	-	-
Gfi1$^{-/-}$ R1	-	-	-
Gfi1$^{-/-}$ R2	-	-	-
Gfi1$^{-/-}$ R3	-	-	-

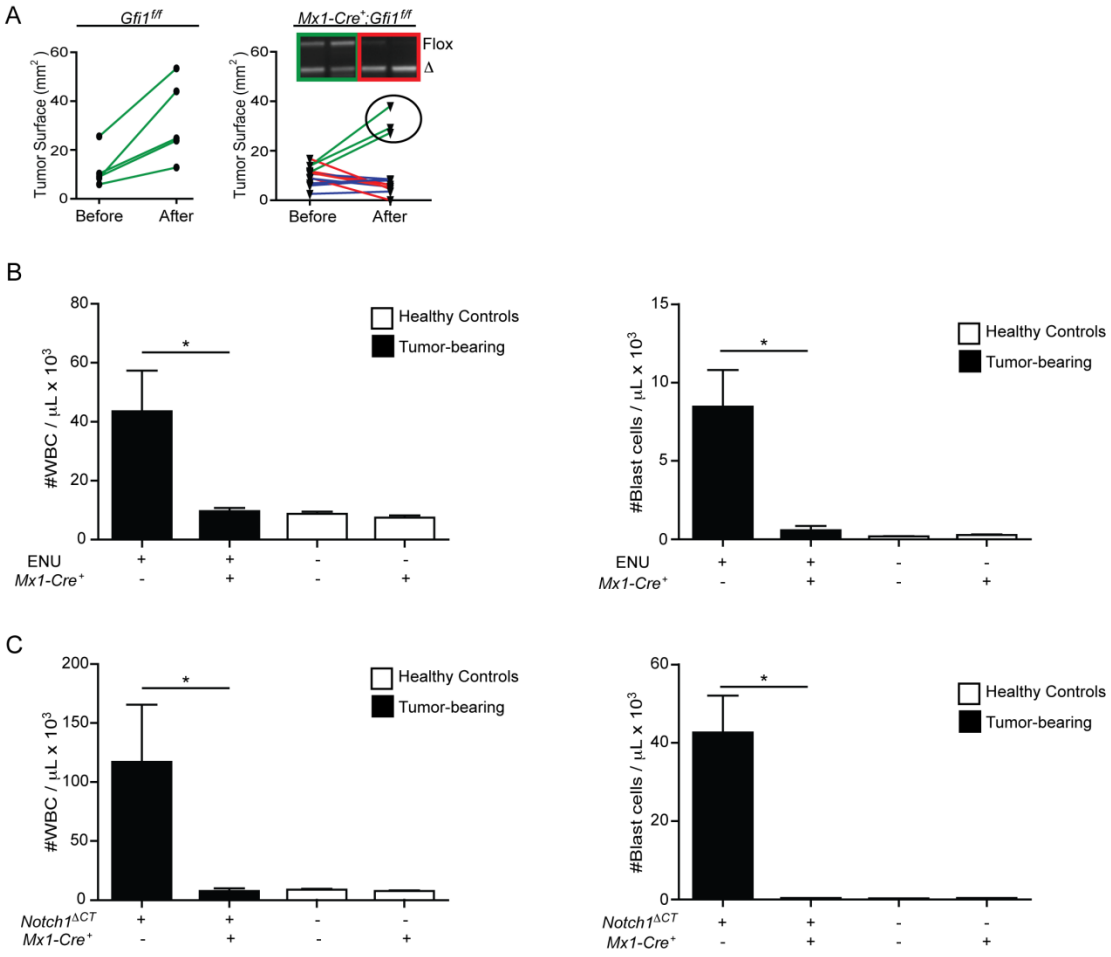
A
Gfi1f/f
Mx1-Cre+:Gfi1f/f
Flox
Δ
Tumor Surface (mm2)
Before
After
B
Healthy Controls
Tumor-bearing
#WBC / µL x 10^3
#Blast cells / µL x 10^3
ENU
Mx1-Cre+
C
Notch1ΔCT
Mx1-Cre+

Figure S2, related to Figure 2. ***Gfi1*** **is critical for T-ALL tumor maintenance**

(A) Tumor surface area (arising from mice described in Figure 2B) was examined by ultrasound before and after pIpC injection in *Gfi1*$^{f/f}$ (left) and *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ (right) mice. Mice in which tumor surface area increased after pIpC treatment are shown in green, those that remained unchanged are in blue, and those that decreased are displayed in red. Insert: PCR analysis of the *Gfi1* locus in representative tumors for *Gfi1* flox and excised (Δ) alleles.

(B) As described in Figure 2B, *Gfi1*$^{f/f}$ (n=6) or *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ (n=4) mice were treated with ENU and subsequently monitored by ultrasound for tumor development. Upon appearance of a mass, mice were injected with pIpC and followed for tumor progression or regression by ultrasound. At the end of observation, the number of white blood cells (WBC, left) and leukemic "Blast" cells (right) in the peripheral blood was determined and compared to results of healthy pIpC injected *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ and *Gfi1*$^{f/f}$ mice (n=3 for both groups).

(C) As described in Figure 2D, *Notch1*$^{\Delta CT}$*;Gfi1*$^{f/f}$ (n=2) or *Notch1*$^{\Delta CT}$*;Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ (n=3) mice were treated with ENU and subsequently monitored by ultrasound for tumor development. Upon appearance of a mass, mice were injected with pIpC and followed for tumor progression or regression by ultrasound. At the end of observation, the number of white blood cells (WBC, left) and leukemic "Blast" cells (right) in the peripheral blood was determined and compared to results of healthy pIpC injected *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ and *Gfi1*$^{f/f}$ mice (n=3 for both groups).

| in all Kaplan-Meier curve plots indicate censored mice.

Mean and ±SEM are shown unless stated otherwise. *p<0.05.

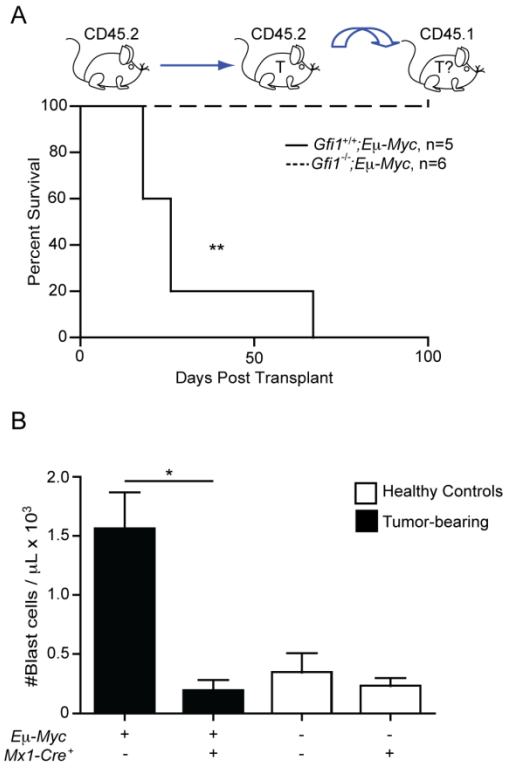

Figure S3, related to Figure 3. ***Gfi1*** **is critical for B-ALL tumor maintenance**

(A) Lymphomas from *Gfi1*$^{+/+}$*;Eμ-Myc* (black line) and *Gfi1*$^{-/-}$*;Eμ-Myc* (dotted line) mice (CD45.2, Figure 3A) were transplanted into sublethally irradiated congenic recipients (CD45.1) which were monitored for tumor progression and host survival.

(B) As described in Figure 3C, *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$*;Eμ-Myc* and *Gfi1*$^{f/f}$*;Eμ-Myc* mice were observed by ultrasound for appearance of B-cell lymphoma. Upon appearance of a mass, mice were injected with pIpC and monitored for tumor progression and survival. At end of observation, the number of leukemic "Blast" cells in the peripheral blood of these mice was determined and compared to results of healthy pIpC injected *Mx1-Cre*$^{+}$*;Gfi1*$^{f/f}$ and *Gfi1*$^{f/f}$ mice (n=3 for each group).

Mean and ±SEM are shown unless stated otherwise. *p<0.05, **p < 0.01.

Table S2, related to Figure 4. GSEA Signatures.

Please see Excel file.

GSEA signatures are displayed in indicated pathways from Gfi1 sufficient (*Gfi1*$^{+/+}$ or *Gfi1*$^{f/f}$) or Gfi1 deficient (*Gfi1*$^{-/-}$ or *Gfi1*$^{\Delta/\Delta}$) T cells, irradiated T cells, ENU-induced tumors, or *Notch1*$^{\Delta CT}$ ENU accelerated tumors.

Depicted GSEA signatures are shown where at least one comparison between matched tissues resulted in an FDR q-value <0.25.

GSEA signatures highlighted in blue represent signatures statistically enriched in DNA damage containing matched tissues, but not in unirradiated T cells.

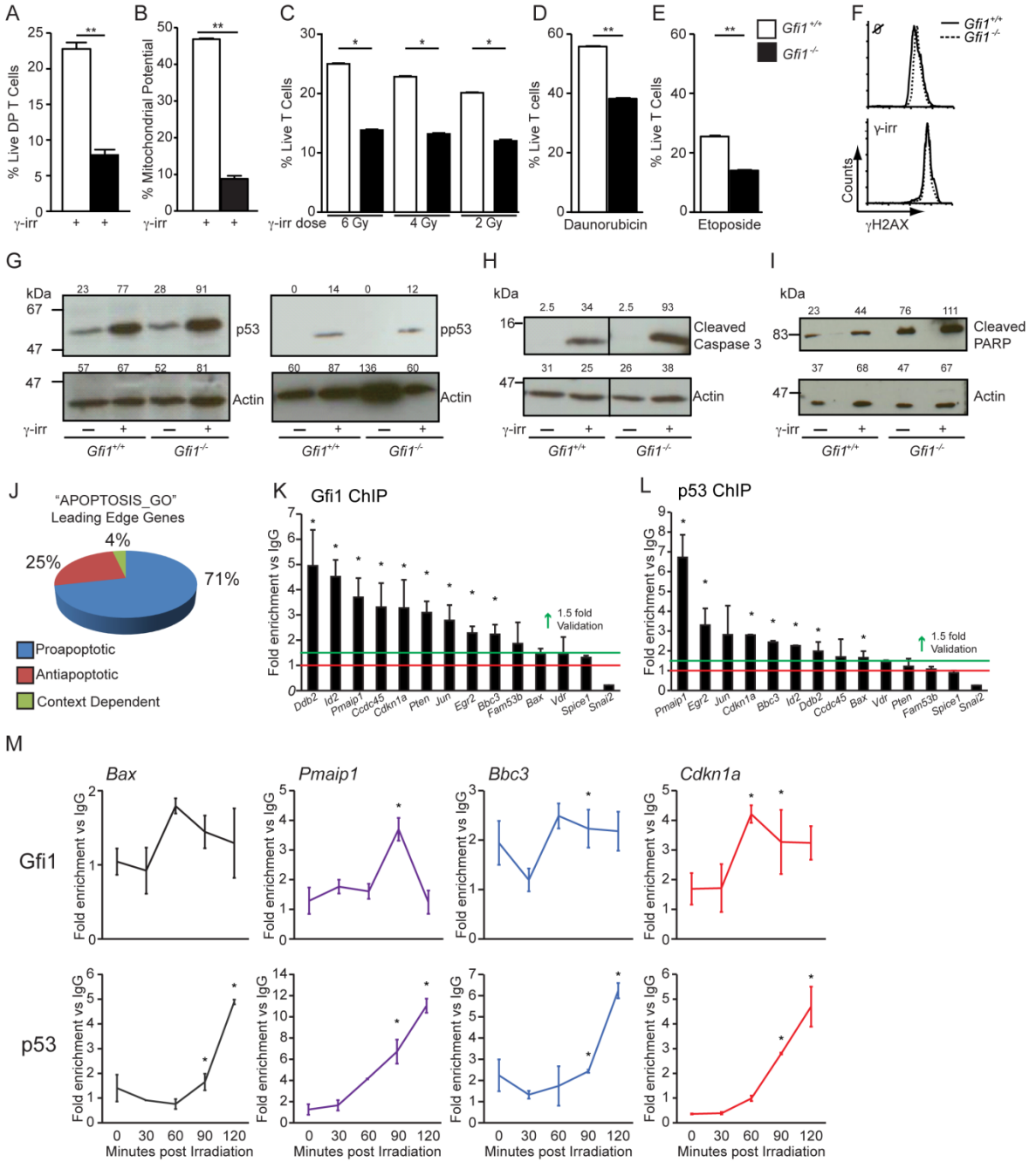
A
% Live DP T Cells
γ-irr
B
% Mitochondrial Potential
C
% Live T Cells
γ-irr dose
6 Gy
4 Gy
2 Gy
D
% Live T cells
Daunorubicin
E
% Live T Cells
Etoposide
Gfi1+/+
Gfi1-/-
F
γ-irr
Counts
γH2AX
G
kDa
p53
pp53
Actin
H
Cleaved Caspase 3
I
Cleaved PARP
J
"APOPTOSIS_GO"
Leading Edge Genes
4%
25%
71%
Proapoptotic
Antiapoptotic
Context Dependent
K
Gfi1 ChIP
Fold enrichment vs IgG
1.5 fold Validation
Ddb2
Id2
Pmaip1
Ccdc45
Cdkn1a
Pten
Jun
Egr2
Bbc3
Fam53b
Bax
Vdr
Spice1
Snai2
L
p53 ChIP
M
Bax
Pmaip1
Bbc3
Cdkn1a
Gfi1
p53
Minutes post Irradiation

Figure S4, related to Figure 5. *Gfi1*$^{-/-}$ thymocytes are more sensitive to apoptosis after p53-indcued DNA damage.

(A) Percentage of AnnexinV negative CD4^{+}CD8^{+} FACS sorted thymocytes from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ mice after *ex vivo* γ-irradiation (n=3 for each genotype from one experiment).

(B) Thymocytes from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ mice were explanted and γ-irradiated. Mitochondrial membrane potential was measured by JC-1 dye fluorescence in the FITC and PE channels by flow cytometry. The percent of thymocytes showing dye fluorescence in both channels is shown (n=3 for each genotype from one representative experiment). Level of mitochondrial potential loss after irradiation was normalized to untreated samples.

(C-E) Percentage of AnnexinV negative thymocytes from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ mice after various doses of *ex vivo* γ-irradiation (C), Daunorubicin (D) or Etoposide (E) treatment (n=3 for each genotype from one experiment).

(F) Flow cytometric quantification of γH2AX in total thymocytes from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ mice pre- and 120 min post- γ-irradiation (6 Gy) from one experiment.

(G) Immunoblot analysis of total cell lysate using antibodies against p53 (left) or phospho-p53 (right) from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ thymocytes pre- and 180 min post irradiation (6 Gy). Numbers indicate densitometric quantification from one experiment.

(H, I) Immunoblot analysis of total cell lysate using antibodies against cleaved Caspase-3 (H) or cleaved PARP (I) from *Gfi1*$^{+/+}$ and *Gfi1*$^{-/-}$ total thymocytes after *ex vivo* pre- and 180 min post γ-irradiation (6 Gy). Numbers indicate densitometric quantification from one experiment.

(J) Pie chart characterizing the Leading Edge genes from the "Apoptosis_GO" GSEA signature from irradiated thymocytes according to their GO biological function.

(K, L) Wild-type thymocytes were irradiated (5 Gy) and chromatin was generated 90 minutes later. ChIP-qPCR was performed on chromatin immunoprecipitated with antibodies against Gfi1

(K) or p53 (L). Values represent the fold enrichment of binding for p53 and Gfi1 to the indicated regulatory regions of the annotated genes compared to the IgG controls from one experiment with three different technical repeats.

(M) Wild-type thymocytes were irradiated (5 Gy) and chromatin was generated 0, 30, 60, 90 and 120 minutes later. ChIP-qPCR was performed on chromatin immunoprecipitated with antibodies against Gfi1 (top) or p53 (bottom) for four of the 14 genes analyzed in (K, L). Values represent the fold enrichment of binding for p53 and Gfi1 to the indicated regulatory regions of the annotated genes compared to the IgG controls from one experiment with three different technical repeats.

Mean and ±SEM are shown unless stated otherwise. *$p<0.05$, **$p < 0.01$.

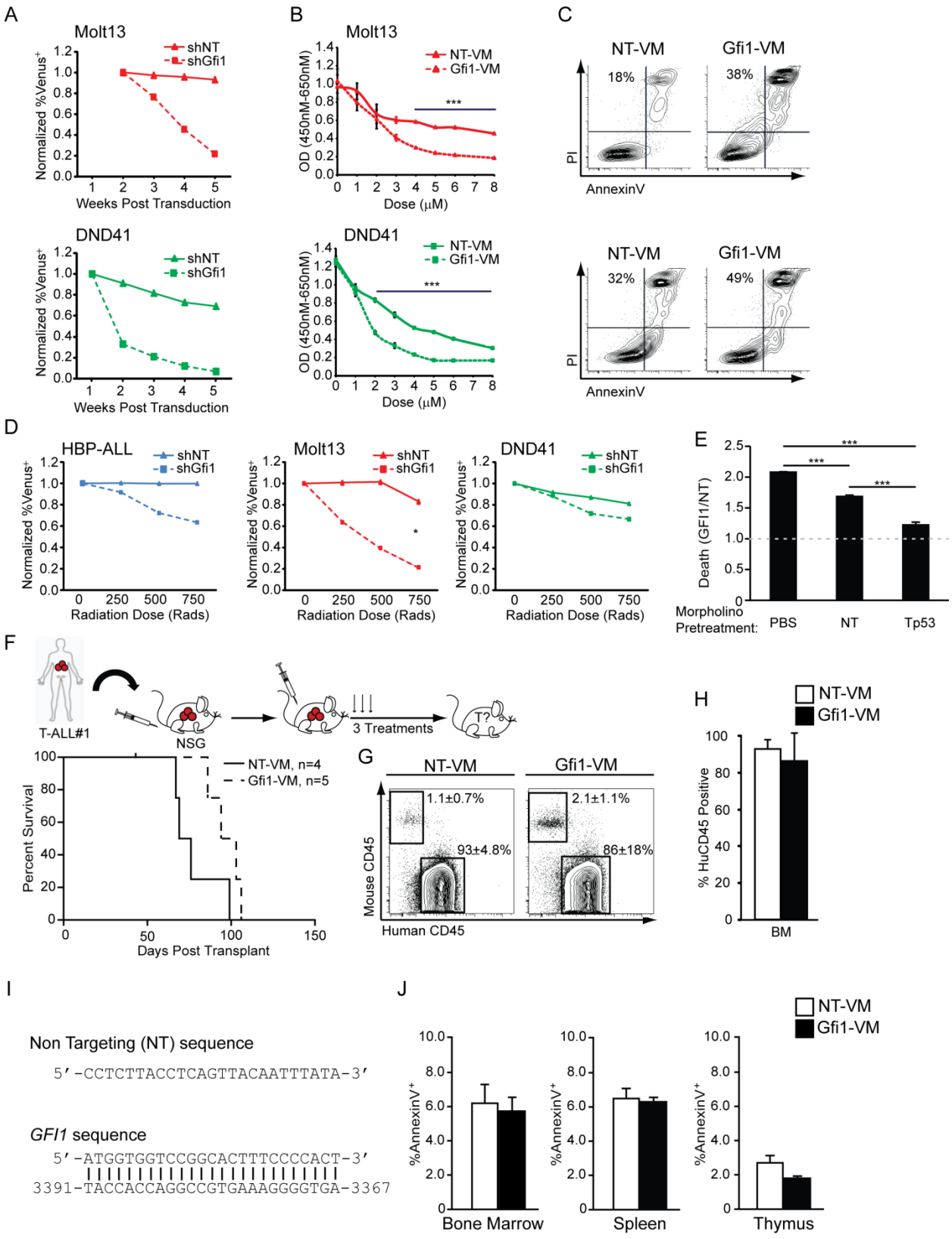
A
Molt13
shNT
shGfi1
Normalized %Venus+
Weeks Post Transduction
DND41
B
Molt13
NT-VM
Gfi1-VM
OD (450nM-650nM)
***
Dose (μM)
DND41
C
NT-VM
Gfi1-VM
18%
38%
PI
AnnexinV
32%
49%
D
HBP-ALL
Molt13
DND41
Radiation Dose (Rads)
E
Death (GFI1/NT)
Morpholino Pretreatment:
PBS
NT
Tp53
F
T-ALL#1
NSG
3 Treatments
T?
NT-VM, n=4
Gfi1-VM, n=5
Percent Survival
Days Post Transplant
G
1.1±0.7%
93±4.8%
2.1±1.1%
86±18%
Mouse CD45
Human CD45
H
% HuCD45 Positive
BM
I
Non Targeting (NT) sequence
5′-CCTCTTACCTCAGTTACAATTTATA-3′
GFI1 sequence
5′-ATGGTGGTCCGGCACTTTCCCCACT-3′
3391-TACCACCAGGCCGTGAAAGGGGTGA-3367
J
%AnnexinV+
Bone Marrow
Spleen
Thymus

Figure S5, related to Figure 6. Loss of Gfi1 in human T-ALL induces apoptosis and tumor regression

(A) Indicated T-ALL cells were transduced with YFP marked shRNA expressing lentiviral vectors targeting Gfi1 (shGfi1, dotted line) or non-targeting control (shNT, solid line). Expression of YFP was measured by FACS 72 hours post transduction, which was set as 1, and subsequent measurements were taken by FACS over a 5 week period and normalized to the first reading, p=0.123 (Molt13), p=0.058 (DND41).

(B) Growth of T-ALL cell lines Molt13 (top) or DND41 (bottom) treated with various doses of Gfi1 (dotted line) or NT (solid line) Vivo Morpholinos (VM) as measured by WST assay after 48 hours of VM treatment.

(C) AnnexinV and PI staining after 16 hours of culture with NT or Gfi1 VM (4 μM) in Molt13 (top) or DND41 (bottom) cell lines. 1 of at least 3 representative experiments is shown (A-C).

(D) T-ALL cell lines were transduced with Venus-marked lentiviral shRNA control (shNT, solid line) or Gfi1 constructs (shGfi1, dotted line), plated and then irradiated at various doses. Cells were analyzed for $Venus^+$ cells the following 3 days. Measurements were normalized to 1 at the first time point (non-irradiated). Graphs display data from 72 hours post irradiation. 1 of 3 different time points is shown.

(E) Molt13 cells were treated for 24 hours with PBS, NT or Tp53-specific VM (4 μM). Media was changed and cells were replated with NT or Gfi1 VM (4 μM). AnnexinV and PI staining was measured 24 hours later by flow cytometry. The ratio of the percent increase in dead cells with Gfi1 compared to NT VM treatment is displayed. 1 of 4 representative experiments using various T-ALL cell lines is shown.

(F) Top: Primary patient T-ALL samples passaged in NSG mice and four days later were treated (i.v.) with 12.5 mg/kg of Gfi1 or NT VM once a week for 3 weeks. Bottom: Kaplan-Meier plot of NT or Gfi1-treated VM treated mice (p=0.07).

(G) Post mortem FACS analysis of bone marrow for human T-ALL cells from above NSG recipient mice.

(H) Graphical representation of (G, n=3 for each).

(I) Sequence of the NT and Gfi1 Vivo Morpholinos.

(J) WT (n=3 for each group) mice were injected with either murine Gfi1-specific or NT control Vivo Morpholino once every other day for a total of 9 injections times. Two days after the last injection, indicated organs were harvested and apoptosis was measured by AnnexinV staining.

| in all Kaplan-Meier curve plots indicate censored mice.

Mean and ±SEM are shown unless stated otherwise. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Experimental Procedures

Mouse strains

The generation of *Notch1*$^{\Delta CT}$, *Gfi1*$^{-/-}$, *Gfi1*$^{f/f}$, *H2*^{k}*-Bcl2-tg, Eμ-Myc, RosaCre*^{ERT2} and *RosaICN*^{LSL} mice have been previously published (Karsunky et al., 2002; Kelly et al., 2007; Kondo et al., 1997; Priceputu et al., 2006; Yang et al., 2004; Zhu et al., 2006).

Tumorigenesis assays

Transplants: Donor mice were primed with 250 mg/kg of 5-flurouracil (5-FU) and BM cells were harvested 72 hours later. Low-density BM was fractionated by Histopaque 1083 (Sigma-Aldrich, St. Louis, MO), cytokine expanded for 48 hours, and then spin infected twice with retroviral supernatants 24 hours apart as previously reported (Horman et al., 2009). Transduction rates were determined by flow cytometry and equal numbers of transduced cells were transplanted into 6-8 week old syngenic recipients after sublethal radiation. ENU: Three to nine-week old mice were injected once a week (for a total length of three weeks) with 100 mg/kg of ENU: ENU-induced BM failure was defined as having a hemoglobin level lower than 8 dg/dL, platelets under 100/fL and leukocytes below 2/fL. MMLV: New-born mice (1-2 days old) were injected with 100 μL of MMLV (PFU $2x10^5$) as described (Schmidt et al., 1996). Secondary transplants were performed in six to ten week old CD45.1 mice after sublethal irradiation (6 Gy) and injected with 10^5 (CD45.2) tumor cells. Engraftment was verified by the presence of $3x10^5$ CD45.2 cells, which were co-injected at the time of transplant.

In vivo deletion of Gfi1 and ultrasound observation

Gfi1$^{f/f}$ or *RosaCre*^{ERT2};*Gfi1*$^{f/f}$ mice were injected (i.p) with 1 mg OHT (Sigma-Aldrich) dissolved in 100 uL of corn oil the first five days following transplantation. *Gfi1*$^{f/f}$ or *Mx1-Cre*$^{+}$;*Gfi1*$^{f/f}$ mice were either injected (i.p) four weeks after the last ENU injection or three days after the transplantation of the tumor cells with 500 mg pIpC (Sigma-Aldrich) seven times every other day. PCR validation of *in vivo* deletion was performed as previously described (Horman et al., 2009). Ultra sound observation was performed on anesthetized mice and thymic tumors were measured using the Visualsonic ultrasound machine and the Vev0770 imaging software (both Toronto, Canada). A tumor was called present if the thymic surface area measured in the horizontal and sagittal plane was larger than 8 mm^2, as average thymic surface of age matched, untreated *Gfi1*$^{f/f}$ control mice is 4 mm^2, and if the tumor exhibited growth of more than 50% during the last two weeks of observation.

In vitro cell culture and treatments

Thymocytes or primary tumors (purity ≥95 % as measured by FACS) were explanted and put in culture at a concentration of $2x10^6$ cells/mL/well (of a 12 well plate) in 10% fetal calf serum (FCS, Hyclone (Waltham, MA, USA), 1640 RPMI (Wilsent, Montreal, Canada)). Cells were either left untreated or irradiated (6 Gy). Six hours after irradiation, viability was measured by either using the AnnexinV staining method or by measuring loss of mitochondrial potential (JC-1 mitochondrial potential kit, Invitrogen) according to manufacturer's protocol. Values were normalized to untreated controls for both AnnexinV staining and mitochondrial membrane potential assays. For DNA damage induction by chemical, Daunorubicin was added at a concentration of (10 µg/mL) and Etoposide at a concentration of (10 µg/mL). Murine T-ALL

cell lines were maintained in 1640 RPMI (Gibco) with 20% FCS, 1% Pen/Strep, 1% L-glutamine and 50 mM β-mercaptoethanol. Human T-ALL cell lines were maintained in 1640 RPMI (Gibco) with 10% FBS (Gibco), 1% Pen/Strep, and 1% L-glutamine. Obatoclax (Selleck Chem.) was dissolved in DMSO and added to T-ALL cultures at indicated doses.

Sequences for Non-Targeting (SHC002, CCGGCAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTGTTTTT) and Gfi1 (TRCN0000020465, CCGGCCAGACTATTCCCTCCGTTTACTCGAGTAAACGGAGGGAATAGTCTGGTTTTT) shRNA were purchased in the lentiviral vector pLKO.1 (Sigma). The eYFP fluorescent marker Venus was cloned into the location of the puromycin resistance gene. Lentiviral transduction levels of T-ALL cells were determined 48-72 hours post transduction by flow cytometry. Subsequent measurements were normalized to the initial reading. Counter-selection of *GFI1*-targeting shRNAs was observed as evidenced by >50% reduction in Venus levels over time.

γH2AX foci

γH2AX foci were detected by using the γH2AX specific antibody from BD (560445). Immunofluorescence and FACS was performed according to manufacturer's manual.

ChIP, ChIP-Seq & RT-PCR

ChIP-Seq ChIP assays were performed as previously described (Forsberg et al., 2000) using a polyclonal anti-Gfi1 (abcam ab21061) antibody. Briefly, $2x10^7$ MLL-ENL immortalized bone marrow progenitor cells were cross-linked in 1 % formaldehyde, lysed and sonicated in fragments of about 150 to 400 bp. Each sample was amplified using the Illumina kit following

manufacturer's instructions and sequenced using the Illumina 2G Genome Analyzer. Sequencing reads were mapped to the mouse reference genome using Bowtie converted to a density plot as described (Wilson et al., 2009), and displayed in UCSC genome browser. ChIP was performed on explanted irradiated thymocytes after treatment using similar procedures as above and an anti-p53 antibody (Fl393, Santa Cruz). To detect p53 methylation, the anti-p53 (mono methyl K372) antibody (ab16033, ABCAM) was used in conjunction with the anti-p53 antibody (DO-1). This antibody also detects murine p53 methylated at K369, which corresponds to K372 in the human p53 (Kurash, JK et al., Molecular Cell, 2008). Real-Time PCR was performed with primers covering the following sites *Bax*: F-CGGCAATTCTGCTTTAACCT, R-CGCCCCCATTATTTCTTCTT; *Bbc3*: F-CTTGTGCCCCAGCTTTCAT, R-GAGTCCCAGGTGCTTCCTTC; *Ccdc45*: F-CGATCTGAGAACCCGTAAGC R-GACAGCAGAGGAGCACTTCC; *Cdkn1a*: F1-CGCTGCGTGACAAGAGAATA, R1-CCTCCCCTCTGGGAATCTAA; F2- TCCTTTTCTGGGAAGTGGTG, R2-AGGTATCCTCTGGGGCTGAT; *Ddb2*: F-GAGGACCTCTGCGACTTTTG, R-CTTTTCCACTCCTTGCTTCG; *Egr2*: F-AGCGTGGGTCAAGAAAGAGA, R-GTAGCCTGGGATAGCAGACG; *Fam53b*: F-GGAAGTAGCACCGCACAAGT, R-GGTCTTGTTCGCCACAGATT; *Id2*: F-AGGCTGACGATAGTGGGATG, R-CTCCAAGCTCAAGGAACTGG; *Jun*: F-GACACTGGGAAGCGTGTTCT, R-ATGGGCACATCACCACTACA; *Pmaip1*: F-CCCAGCAATGGATACGATCT, R-GAGGACGAGTCCTGCTCAAC; *Pten*: F-ATGTGGCGGGACTCTTTGT, R-TCCGAGAGGAGAGAGCTGAG; *Snai2*: F-ATGAGCAGCCCATTTTGAAC, R-AACCAATCACAGCCAAGAGG; *Spice1*: F-CTCCACTGGGAGGTAGCAAG, R-

ACCTCCACATACCGCACTTC; *Vdr*: F-GTTAAGGACGTTGGCTGCTC, R-AAGGTGTGTGGGACCTCTTG

Reporter assay and immunoprecipitation

Reporter and immunoprecipitation assays were performed as previously described (Khandanpour et al., 2010a; Khandanpour et al., 2010b).

Xenograft transplants and Morpholino Treatment

T-ALL #1 was immunophenotyped as a $CD5^{+}$, $CD10^{(dim)}$, surface $CD3^{(dim)}$, $CD4^{+}$, $CD8^{+}$, $CD7^{+}$, cytoplasimic $CD3^{+}$, TdT^{+}, $CD38^{+}$ tumor and found to have a t(11;14) translocation by FISH. T-ALL #2 was classified as FABL2 thymic T-ALL and immunophenotyped as a $CD1a^{+}$, $CD2^{+}$, cytoplasmic $CD3^{+}$, $CD7^{+}$, $CD14^{(dim)}$, $CD20^{(dim)}$ tumor. NT, Gfi1 or Tp53 (5'-TAGGATCTGACTGCGGCTCCTCCAT-3') Vivo Morpholinos were added to T-ALL cell cultures at 4 μM unless otherwise stated.

Sequence generation and dosing of morpholinos:

Vivo Morpholino sequences were designed by the experts at Gene Tools, LLC (Philomath, OR) to specifically bind to the translation start site of *GFI1*. Oligo sequences were next Blasted (NCBI) to verify sequence specificity and validate that other known genes were not inadvertently targeted. Downregulation of *GFI1* mRNA and protein was also verified in various human T-ALL cell lines (Figure 6 and data not shown). Control non-targeting (NT) Vivo Morpholinos were also designed by the experts at Gene Tools with a sequence that does not target any known

mammalian gene sequence. All mice received approximately 60 µL of a 500 µM Vivo Morpholino solution (i.v.). Dosing varied between 3 to 9 injections. Blood volume of an average 0.020 kg mouse was estimated to be 1.2 mL, resulting in a 25 nM dose (per injection).

The sequence for targeting murine *Gfi1* is: TCTTGACCAGGAATGAGCGCGGCAT.

Gene expression arrays and analyses:

Gene expression arrays were performed according to published procedures (Valk et al., 2004). Microarray data was analyzed using GeneSpring (Agilent Technologies). Published microarray data for T-ALL patients was downloaded and analyzed with GeneSpring software. Data was imported into GeneSpring and the median of each probe was normalized to 1. Gene expression data was filtered for probesets of published Notch1 target genes which were in turn used for unsupervised hierarchical clustering of samples. Normalized expression values were exported, averaged by group (ie. Notch1 target gene expression, *NOTCH1* mutation status, or clinical diagnosis) and then graphed. GSEA was performed comparing each tumor or tissue type to its *Gfi1*$^{-/-}$ counterpart against all curated gene sets in the Molecular Signature Database.

Supplemental References

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