

## Supplemental Figure Legends

### **Figure S1. Doxycycline suppresses transgene expression from the *Col1a1***

**promoter during neonatal stages.** (A) Gating strategy to assess expression of an H2B-GFP reporter that is targeted to the same *Col1a1* locus as the *MLL-ENL* transgene. (B) Percentages of GFP+ HPCs when neonatal mice were maintained on doxycycline from conception through three weeks after birth, or when doxycycline was withdrawn at P0.

### **Figure S2. Adult *MLL-ENL* induction does not result in efficient leukemogenesis.**

(A) Spleen weights of surviving control and Tet-OFF-*MLL-ENL* mice at >1 year after *MLL-ENL* induction in adulthood. (B-D) Histology of representative spleens from surviving control and Tet-OFF-*MLL-ENL* mice. A representative leukemic spleen was included for comparison. Scale bars indicate 100  $\mu$ M. (E) Expression of the *MLL-ENL* transcript at the indicated ages when doxycycline was withdrawn 8 weeks after birth. The data are normalized to the mean expression in mice that never received doxycycline. (F) Western blot for the MLL N-terminus in HPCs and pGMs from mice at P14 and 8 weeks old. An MLL-ENL AML specimen was also included, as was an AML specimen driven by an MLL-AF9 retrovirus.

### **Figure S3. Gating strategies and analysis of doxycycline treated P0 mice.** (A)

Gating strategies for HSCs, HPCs, pGMs and GMPs. (B) HSC, HPC, pGM and GMP numbers in P0 mice that were treated with doxycycline throughout gestation. Unlike

mice that did not receive doxycycline (Fig. 3), these populations were unaffected in Tet-OFF-*MLL-ENL* mice, confirming that the doxycycline can suppress transgene activity *in utero*.

**Figure S4. Gating strategy for BrdU assays.** Representative gates are shown for E18.5 fetal and 8-week-old adult HSCs (A, B) and E18.5 fetal and 8-week-old adult HPCs (C, D).

**Figure S5. Chemotherapy and culture stress do not induce transformation or enhance *MLL-ENL* target gene expression, respectively.** (A) Survival data in tabular form for control and tet-OFF-*MLL-ENL* mice that were maintained on doxycycline until 6 weeks after birth and then treated with PBS, 5-FU or plpC 6 weeks later. All of the mice survived until the experiment was discontinued, 8-12 months after treatment, without developing AML. (B) *Hoxa9*, *Hoxa10* and *Mecom* expression in HPCs that were isolated directly from adult mice, 4 months after doxycycline was discontinued, or that were isolated and cultured for 48 hours. Error bars reflect standard deviations. N=4-6, \*\* $p < 0.01$ , \*\*\* $p < 0.001$  by two-tailed Student's t-test.

**Figure S6. *Lin28b* suppresses *MLL-ENL*-driven AML initiation, but it does not enhance or impede growth of transformed AML cell lines.** (A) Kaplan-Meier survival curves for recipients of EGFP, LIN28B, IGF2BP2, IGF2BP3 and GEM expressing progenitors (n=5 per group). (B) K562 and MOLM14 cells were transduced with EGFP control or LIN28B-P2A-EGFP expressing lentiviruses. Percentages of EGFP positive

cells were measured at day 0 (2 days after infection) and day 3. Data show the percentages of EGFP-positive cells at days 3 and 6 relative to day 0.

## **Supplemental materials and methods**

### *Lentiviral transduction of HPCs and transformed AML lines*

cDNAs to express Enhanced Green Fluorescent Protein (EGFP), LIN28B-P2A-EGFP, IGF2BP2-P2A-EGFP, IGF2BP3-P2A-EGFP and GEM-P2A-EGFP were cloned into the TetO-FUW-oct4 lentiviral vector (Addgene, 20323),<sup>1</sup> in place of the *Oct4* cDNA, for expression in Tet-OFF-*MLL-ENL* HPCs. cDNAs to express EGFP and LIN28B-P2A-EGFP were cloned into pLL-U2G<sup>2</sup> for expression in MOLM14 and K562 cells. To generate viral particles, 293T cells were transfected with the lentiviral plasmid and pRRE, VSVG and RSV-Rev helper plasmids. TransIT-293 reagent (Mirus Bioscience) was used for transfection according to manufacturer protocols. Viral particles were collected from the supernatant and concentrated with Lenti-X (Clontech). Titers were determined by re-infecting 293T cells at limiting doses. Viral stocks were stored at -80°C.

To transduce Tet-OFF-*MLL-ENL* progenitors, 20,000 Lineage<sup>-</sup>c-kit<sup>+</sup> (LK) cells per recipient were isolated from E16.5 livers and transduced by spinoculation. Retronectin (Clontech) was loaded onto 96 well plates per manufacturer instructions. Lentivirus was then added, and the plates were centrifuged at 2000xG for 2 hours at 32°C to bind virus to the retronectin. The supernatant was removed and replaced with 100 µL per well of Stemspan (Stem Cell Technologies) supplemented with Stem Cell Factor (Peprotech, 10 µg/mL), Thrombopoietin (Peprotech, 10 µg/mL) and penicillin/streptomycin (Gibco,

1:100). LK cells were added in an additional 100  $\mu$ L of media and then centrifuged for 10 minutes at 1500 RPM at 32°C. Cells for each recipient were transduced separately so that each recipient would develop leukemias with distinct viral insertion sites. The cells were incubated at 37°C overnight and transplanted into lethally irradiated CD45.1 mice along with 300,000 CD45.1 bone marrow cells for radioprotection. Recipient mice were maintained on normal chow (no doxycycline) to allow expression of the *MLL-ENL* transgene and the lentiviral cDNAs.

To transduce MOLM14 or K562 cells, supernatants from 293T cells were transferred directly into the MOLM14 and K562 culture media. Dose curves were performed so that ~20% of cells were EGFP positive at 2 days post-infection. Cells were then cultured for an additional 6 days and reanalyzed for EGFP expression.

#### *Antibodies for flow cytometry*

All antibodies were from Biolegend except as indicated: CD150 (TC15-12F12.2), CD48 (HM48-1), Sca1 (D7), c-kit (2B8), Ter119 (Ter-119), CD3 (17A2), CD11b (M1/70), Gr-1 (RB6-8C5), B220 (RA3-6B2), CD8a (53-6.7), CD2 (RM2-5), CD45.1 (A20), CD45.2 (104), CD127 (A7R340) and CD16/32 (93). Lineage stains for all experiments included CD2, CD3, CD8a, Ter119, B220 and Gr1. When HSCs, HPCs, pGMs and GMPs were isolated for transplantation or RNA collection, c-kit<sup>+</sup> cells were enriched prior to sorting by selection with paramagnetic beads (Miltenyi Biotec).

### *Western blot*

HPCs and pGMs were isolated from P14 neonates or 8-week-old adult mice. Bone marrow was collected from the hindlimbs and spines, and 100,000 cells were sorted for analysis. AML cells were collected from mice with MLL-ENL or MLL-AF9 driven leukemias. Cells were sorted into PBS + 0.1% BSA. They were pelleted, resuspended in 10  $\mu$ L of PBS and subsequently precipitated in 10% trichloroacetic acid. The precipitates were washed twice with acetone and resuspended in solubilization buffer (9M urea, 2% Triton X-100, 1% DTT). LDS sample buffer (Thermo) was subsequently added to 1x final concentration and the specimen was heated at 70C for 10 minutes. Samples were run on 4-12% NuPage Tris-acetate gels (Thermo) and transferred overnight to PVDF. Westerns were blocked and incubated with primary antibodies. Equivalent results were obtained with monoclonal antibodies from Millipore (clone N4.4, 05-765) and Cell Signaling Technologies (clone D2M7U, 14689) to the MLL N-terminus. Membranes were washed with TBS + 0.05% Tween, incubated with HRP-conjugated secondary antibodies (Cell Signaling) and developed with the SuperSignal Femto kit (Thermo). Beta actin was used as a loading control (Santa Cruz, sc-47778).

### *BrdU incorporation assays*

BrdU (Sigma) was diluted to 5 mg/ml in PBS and administered by IP injections (100 mg/kg/dose) every 8 hr beginning 24 hr prior to bone marrow harvest. For the E18.5 analyses, injections were given to the pregnant mothers. HSCs were stained (CD150-PE, CD48/Lineage – FITC, CD117-biotin, Sca1-percpCy5.5) and enriched by

CD117 (c-kit) selection with paramagnetic beads (Miltenyi Biotec, Auburn, CA). BrdU incorporation was then measured by flow cytometry using the APC BrdU Flow Kit (BD Biosciences). DAPI was used to measure DNA content.

### *5-FU and plpC survival analyses*

Control and tet-OFF-*MLL-ENL* mice were maintained on doxycycline chow from conception until 6 weeks after birth. They were then transitioned to normal chow to induce *MLL-ENL* expression. Six weeks later, they were treated with vehicle (PBS), a single dose of 5-FU (150 mg/kg; Sigma) or three doses of plpC (10 µg/dose every other day; GE Healthcare). The mice were then monitored longitudinally for the time periods indicated in Figure S5A. None of the mice developed AML or died during this evaluation period.

### *qRT-PCR analyses*

Control and tet-OFF-*MLL-ENL* mice were maintained on doxycycline chow from conception until 6 weeks after birth. They were then transitioned to normal chow to induce *MLL-ENL* expression. At 4 months after doxycycline withdrawal, HPCs were isolated by flow cytometry. RNA was isolated from 10,000 HPCs. An additional 20,000 HPCs were cultured in Iscove's media supplemented with 2% fetal bovine serum, IL-3 (10 ng/mL), FLT3 ligand (50 ng/mL), SCF (10 ng/mL) and TPO (10 ng/mL). All cytokines were purchased from Peprotech. The cultured cells were isolated 48 hours later and RNA was purified with the Qiagen RNAeasy micro-plus kit. cDNA was prepared with Superscript III reverse transcriptase (Thermo) and a mix of oligo-dT and random

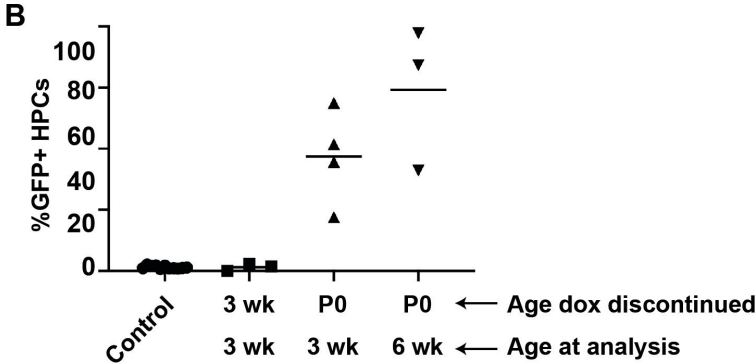
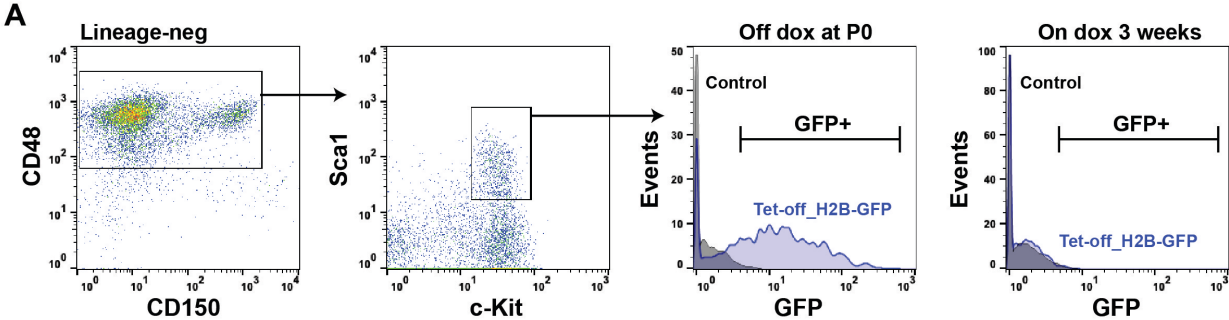
hexamer primers. Quantitative RT-PCR was performed with taqman probes for each indicated gene (Thermo) and normalized to  $\beta$ -actin expression by the  $\Delta\Delta$ CT method.

## Supplemental References

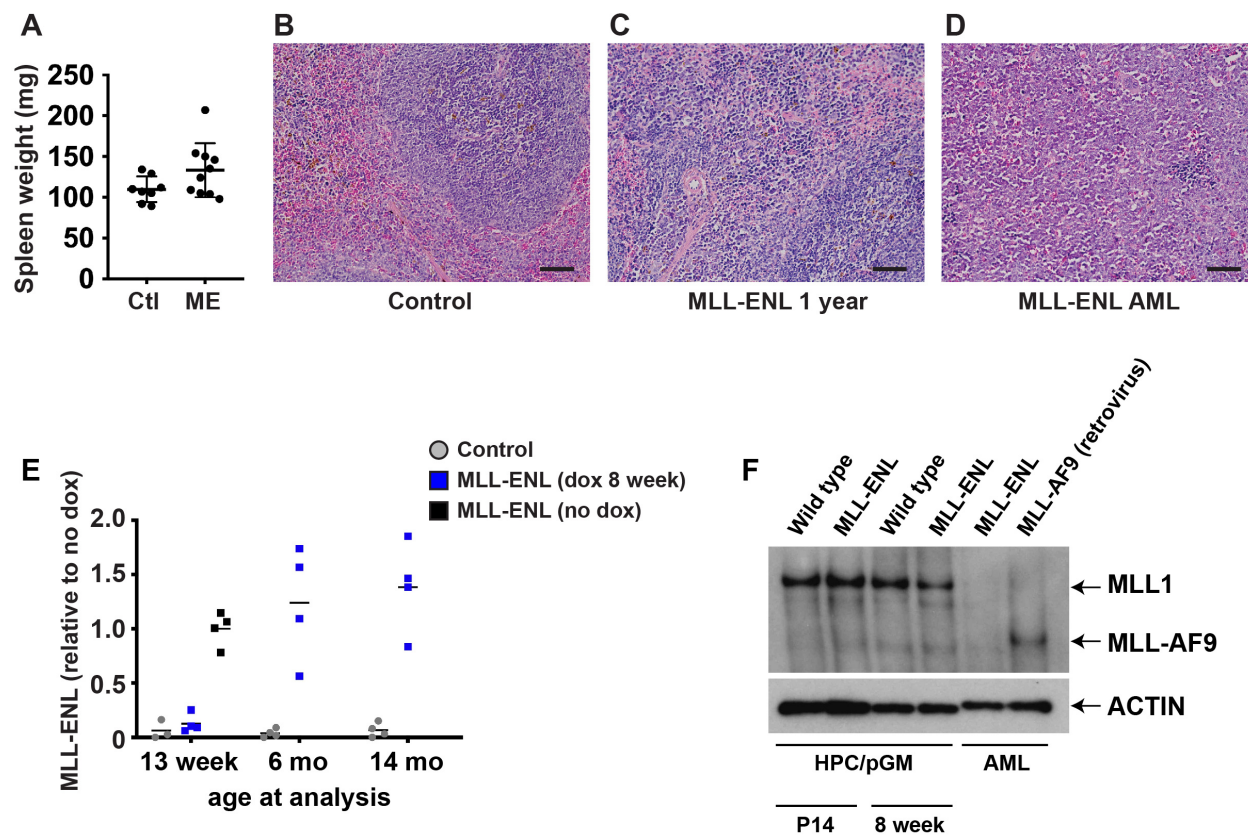
1. Brambrink T, Foreman R, Welstead GG, et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. *Cell Stem Cell*. 2008;2(2):151-159.
2. Dann CT, Alvarado AL, Hammer RE, Garbers DL. Heritable and stable gene knockdown in rats. *Proc Natl Acad Sci U S A*. 2006;103(30):11246-11251.



Supplemental Figure S1

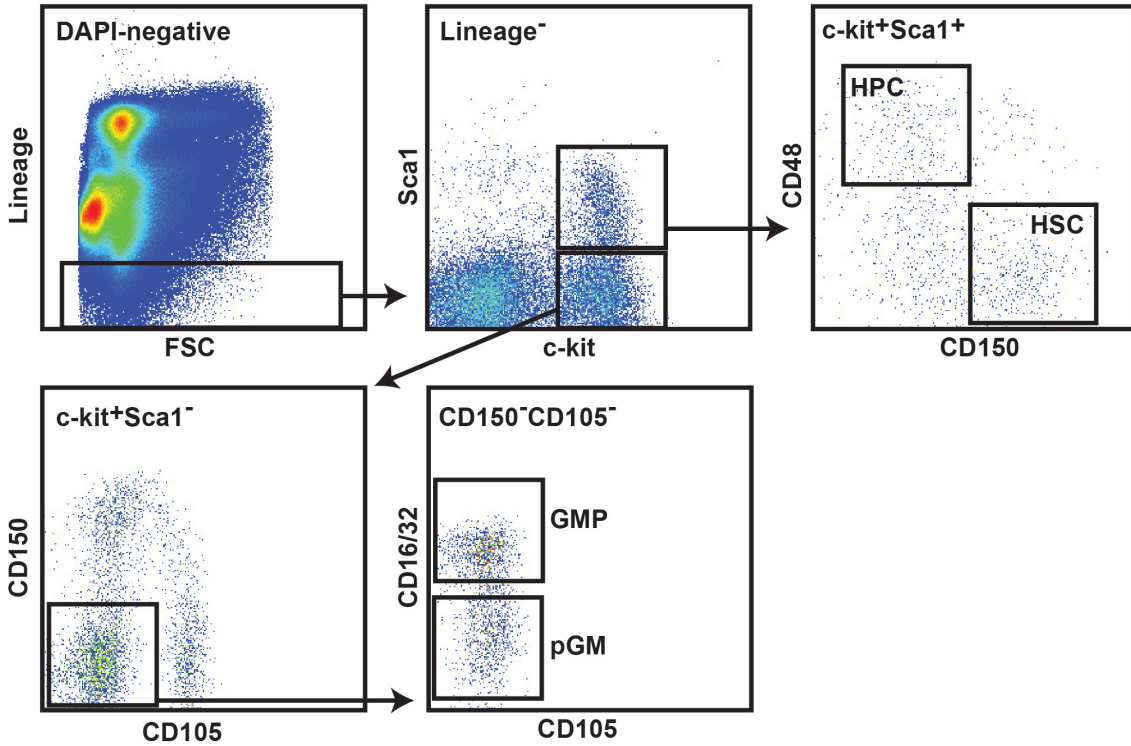


## Supplemental Figure S2

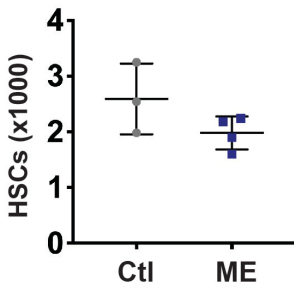


# Supplemental Figure S3

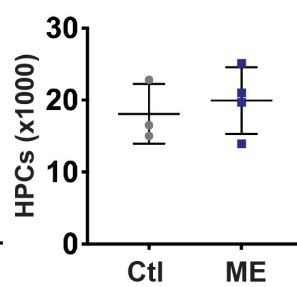
**A**



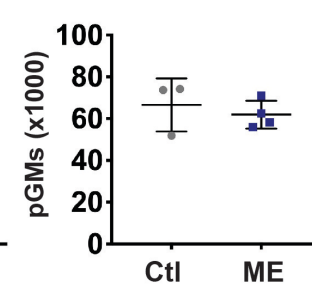
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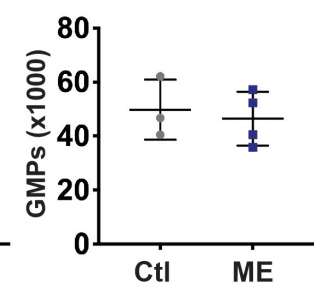
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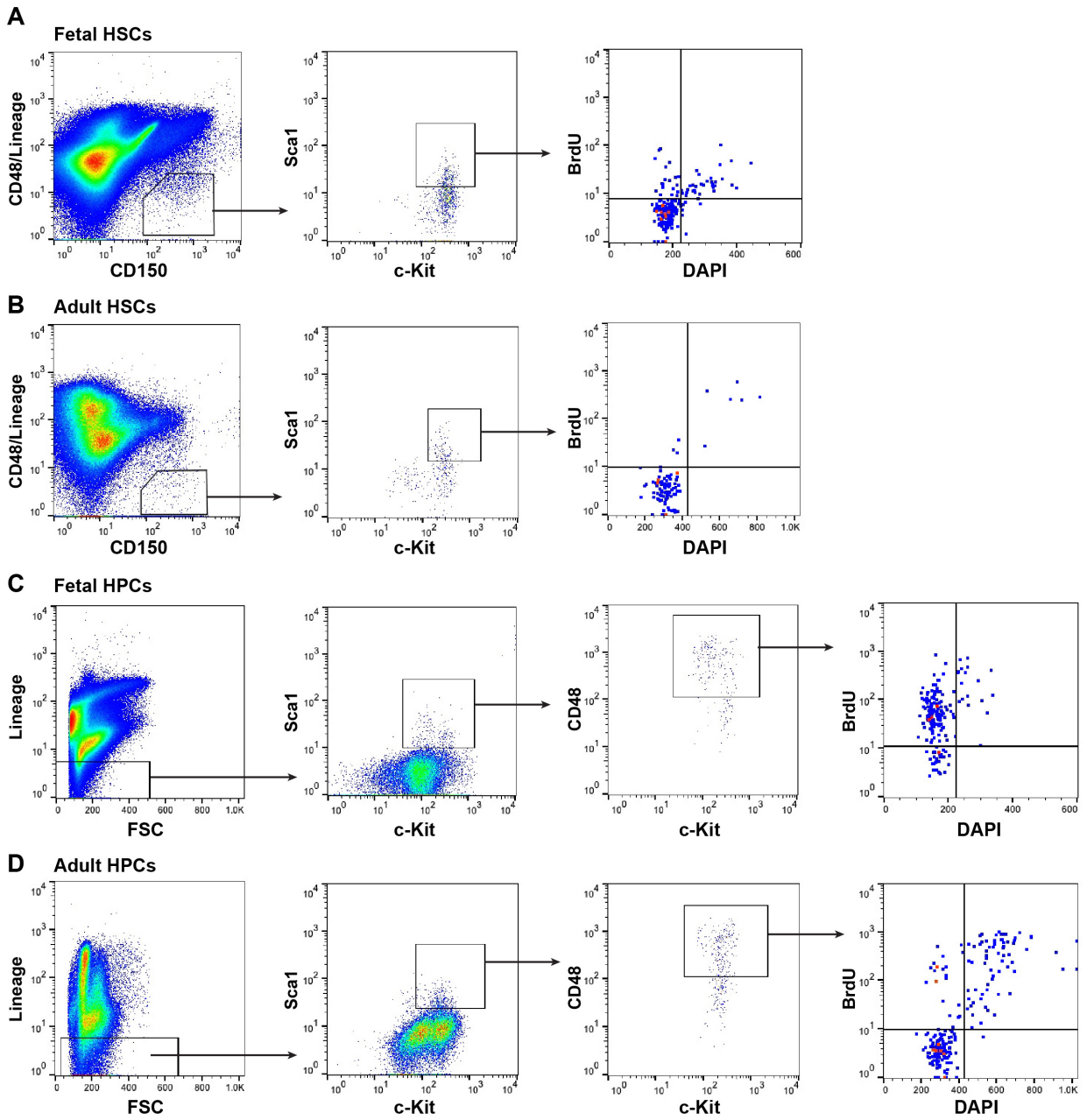
**D**



**E**



# Supplemental Figure S4

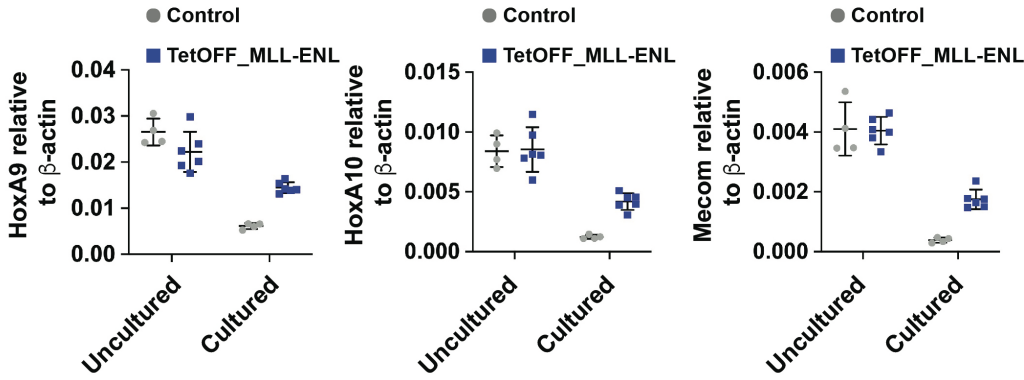


# Supplemental Figure S5

**A**

	<u>Evaluation after treatment (mo)</u>	<u>Percent alive</u>	<u>N</u>
Control PBS	10-11	100	6
Control 5-FU	10-12	100	12
MLL-ENL PBS	10-11	100	7
MLL-ENL 5-FU	10-12	100	10
MLL-ENL plpC	8	100	5

**B**



# Supplemental Figure S6

