SUPPLEMENTAL METHODS

Homing and Lodging

Cre+;Cdc42FL-MA9 leukemia cells were incubated for 48hrs with tamoxifen to delete Cdc42 alleles (KO), or with ethanol control to leave floxed Cdc42 alleles intact (FL). Approximately 5.7 X 10^6 cells were injected via tail vein into mice. Irradiated mice served as recipients for homing assay to quantify cells able to migrate to the marrow compartment. Non-irradiated mice served as recipients for lodging assay to quantify cells able to localize within the intact microenvironment. Mice were sacrificed after 16hrs and flow cytometry was performed on bone marrow (BM) to measure leukemic cell burden.

Genotyping and Protein Expression Analysis

Genotyping protocols were described previously.[12] Immunoblotting was performed with primary antibodies for Cdc42 (BD), Rac1 (Millipore), Bcl-xL (BD), and ß-actin (Sigma). Band intensity was measured using LabWorks software (UVP, LLC). Active GTP-bound Rac1 and Cdc42 was detected using GTPase pull-down assay as previously described.[5]

Imaging Flow Cytometry for Polarity Analysis

Cells were fixed with 4% PFA at RT for 20 mins, then washed with 500 µl PBS. Working quickly, 500 µl of 50% ice cold acetone (1:1 with ddH20) was added dropwise, then cells were spun down for 20 seconds at full speed in table top centrifuge. Supernatant was aspirated, and cells were suspended by adding 500

µl 100% ice cold acetone dropwise. Cells were permeabilized by adding 0.1% Triton X-100 for 10 mins at RT, then blocked by adding 5% donkey serum in PBS for 1 hr at RT. Cells were incubated with 1° antibody (Rat anti-tubulin, Abcam ab6160) at 1:100 in PBS overnight at 4°C, then labeled with 2° antibody Cy3 (Donkey anti-rat, Jackson 712-166-150) at 1:500 for 1 hr RT in PBS with 5% donkey serum. DAPI stain was added 1:100 for 5 mins at RT, then washed, and imaging flow cytometry was acquired on ImageStream (Amnis) at 60X EDF. Analysis was performed using IDEAS 6.2 software. GFP+ cells were used as an apolar control given the even distribution of the fluorophore throughout the cell. Aspect ratio intensity (minor axis intensity/major axis intensity) of tubulin was calculated for mitotic cells. This assigns scores based on the degree to which the fluorophore is displaced from the central axis of the cell and disproportionately concentrated in a quadrant. Cells were considered polar if the aspect ratio intensity score was <0.7, the apolar threshold set by GFP+ cells.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure S1. (A) Loss of Cdc42 (KO) decreased homing of GFP+ MA9 leukemia cells to the bone marrow (BM) compartment. (B) Cdc42KO MA9 cells also had decreased lodging into the intact BM microenvironment.

Supplemental Figure S2. (A) PCR analysis confirmed the expected genotypes in MA9 leukemia cells upon inducible deletion of Cdc42. PCR analysis confirmed deletion of Cdc42 in TAM treated recipients of fully-transformed Cre+;Cdc42FL-

MA9 cells, and did not show emergence of clones that escaped deletion. (B) Secondary recipients of Cdc42KO-MA9 cells died with identical latency as Cdc42FL-MA9 control recipients, suggesting emergence of clones that compensate for absence of Cdc42.

Supplemental Figure S3. Cdc42 deficient LICs contain elevated Rac1 activity. Lysates were made from bone marrow and splenocytes from Cdc42KO-MA9 leukemia mice, with samples from undeleted Cdc42FL-MA9 leukemia mice serving as controls. Lysates were incubated with (GST)-PAK1-PBD beads to pull-down GTP-bound, active Cdc42 and Rac1. Cdc42-GTP and Rac1-GTP levels were normalized to total Cdc42 or Rac1 protein, respectively, and total Cdc42 and Rac1 was normalized to B-tubulin.

Supplemental Figure S4. (A) Explanted MLL-AF9 knock-in (MA9KI) ROSA26-CreERt2 tumors with either wild-type Cdc42 alleles (WT) or floxed Cdc42 alleles (FL) were plated in methylcellulose with 4-OHT (TAM) vs ethanol vehicle control (CON). MA9KI FL TAM cells where Cdc42 is deleted show decreased CFU, consistent with the findings from the retroviral MA9 cell lines. Data is representative of 2-4 repeat experiments. (B) Cre+;Cdc42FL-MA9KI cell lines were injected into congenic recipient mice that were subsequently treated with tamoxifen to induce Cdc42 deletion (KO, N=3), versus corn oil control (WT, N=3). Animals in the Cdc42KO-MA9KI group showed prolonged survival ($p<0.03$), consistent with the effect seen in the retroviral MA9 model.

Supplemental Figure S5. Cre+;Cdc42FL-MA9 leukemia cells were cultured in presence of 4-OHT to induce deletion of Cdc42 in vitro. (A) Floxed Cdc42 alleles are completely deleted by 36 hrs. (B) Cdc42 protein expression is lost by 48 hrs.

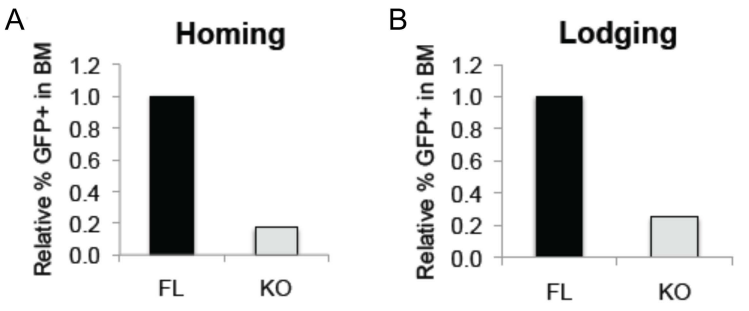
Supplemental Figure S6. Cre+;Cdc42FL-MA9 leukemia cells were transduced with retroviral vector to express Bcl-xL, or empty vector control (MIEG3), then treated with 4-OHT to induce Cdc42 deletion (KO), vs vehicle control (WT). Immunoblot demonstrates the resulting expression level of Bcl-xL.

Supplemental Figure S7. Cre+;Cdc42FL-MA9KI were treated with EtOH (WT) vs 4-OHT (KO) to induce Cdc42 deletion. Cells were co-stained for tubulin and DAPI, then analyzed on ImageStream. Aspect ratio intensity values were calculated using the IDEAS analysis software to determine degree of polarization of tubulin for each mitotic cell. GFP+ cells, which have uniform distribution of the fluorophore, were used apolar controls to determine a threshold value for scoring cells as polar vs apolar. (A) Representative images demonstrate aspect ratio intensity values correlating with tubulin polarity. (B) Distribution of aspect ratio intensity values are shown highlighting 25/50/75 quartiles and the threshold value for polarity (<0.7) and apolarity (>0.7). Cdc42KO-MA9KI cells have increased tubulin aspect ratio intensity values compared to WT MA9KI controls, indicating decreased polarity at day 3 and day 8 post-deletion of Cdc42 ($p<0.0001$).

Supplemental Figure S8. TRMPVIR retroviral vector marked by Venus allows Tet-inducible expression of a miR-30 based shRNA marked by dsRed. (A) Western blot confirmed knockdown of Cdc42 by two distinct inducible shRNA sequences. (B) Immunoblot demonstrates efficient knockdown of Cdc42 by lentiviral shRNA vectors. Venus fluorescent protein was subcloned in place of the puromycin resistance gene cassette in the plko.1 backbone to mark expression shRNA 471 in the patient AML sample xenograft studies.

Supplemental Figure S9. 2016-7 and 2016-9 are two distinct patient AML samples bearing *MLL* (*KMT2A*) gene rearrangements t(9;11) and t(6;11), respectively. These samples were transduced with lentiviral vectors for targeted knockdown of CDC42 (sh471), vs non-targeting control (NT), marked by Venus fluorescent protein. (A) CDC42 knockdown blocked leukemia growth, as evidenced by loss of Venus+ cells in liquid culture. (B) Bone marrow Venus+ graft was analyzed 4 weeks after transplantation into NSGS mice to show significantly reduced disease in the CDC42 knockdown group (N=8) compared to NT control (N=7).

Figure S1



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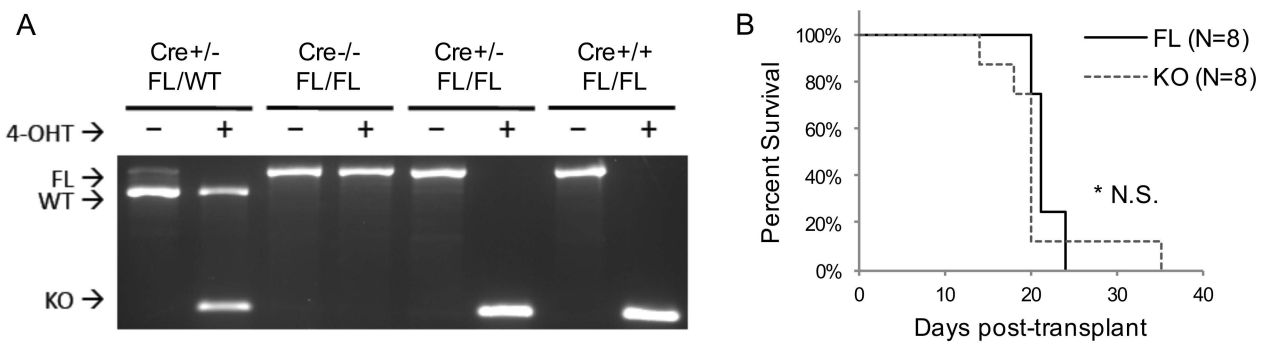

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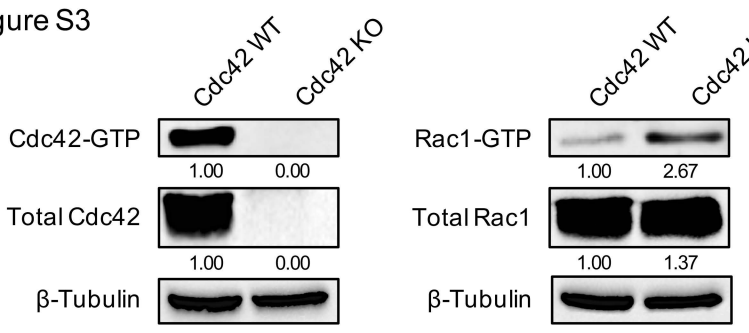

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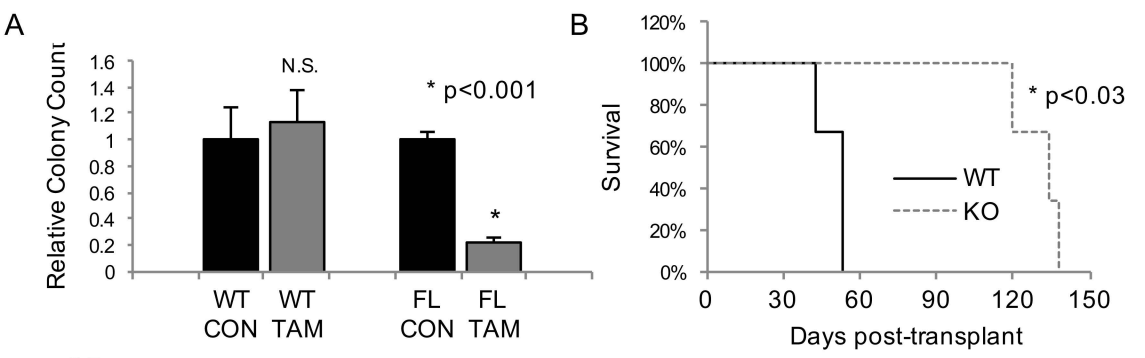

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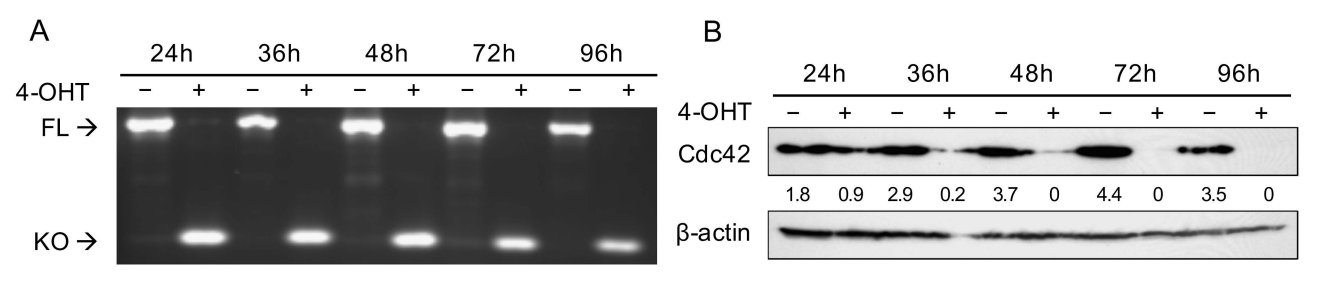
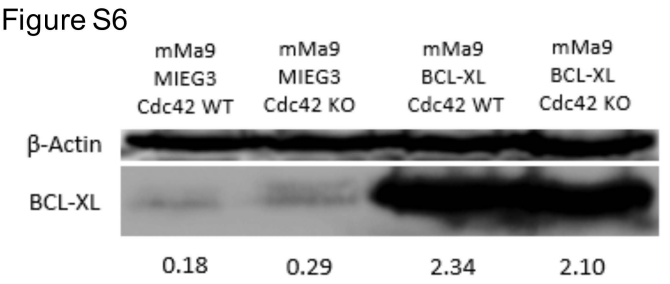


Figure S6



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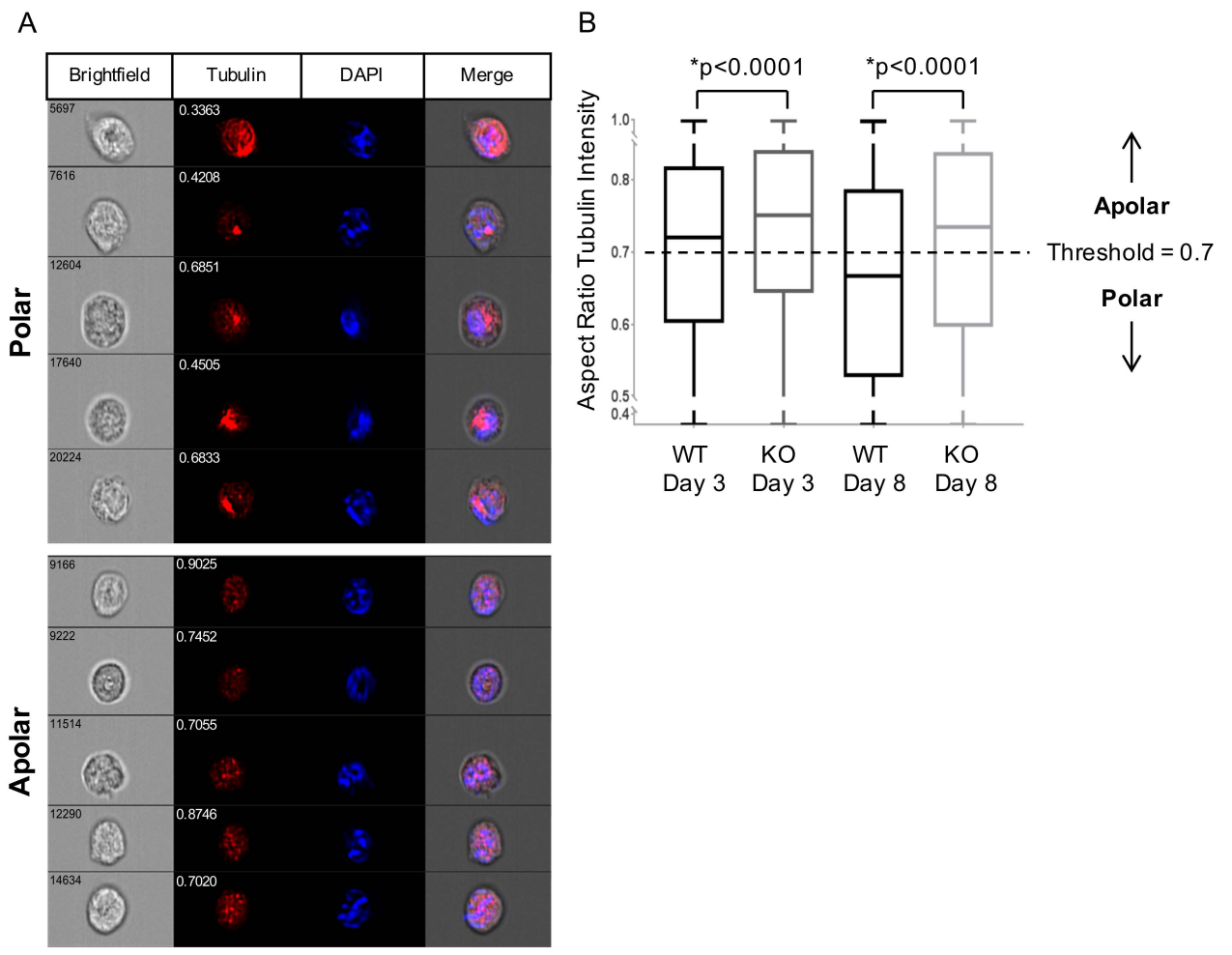

Figure S8

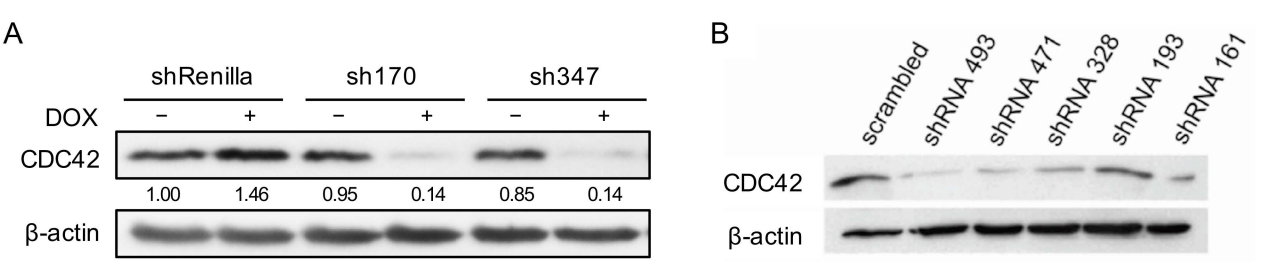

Figure S9