Valerio et al. Figure S1

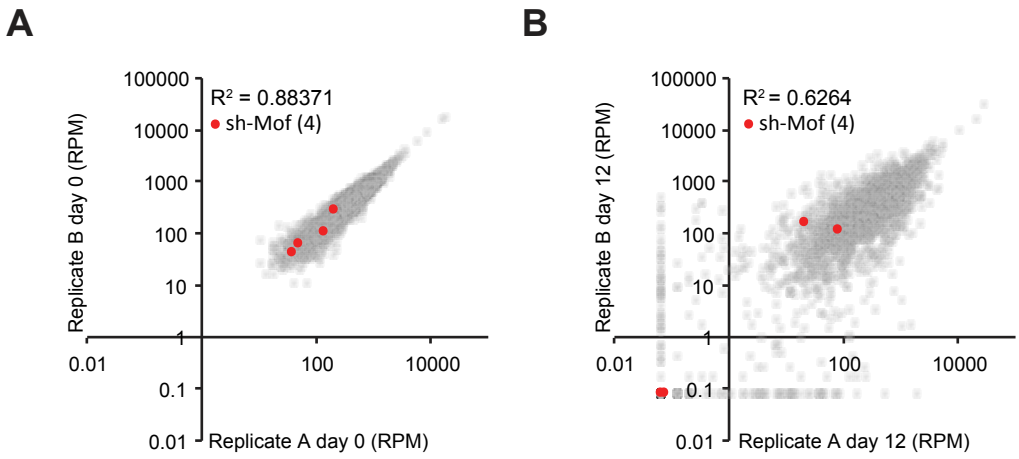



**Figure S1. Chromatin regulator focused RNAi screen in *MLL-AF9* leukemia identifies *Mof* as a key regulator. Related to Figure 1.**

(A) A chromatin regulator focused shRNA library screen was performed in murine primary *MA9* monoclonal leukemia cells. Plotted is the high throughput sequencing (HiSeq) normalized read-count (RPM) for replicate A (x-axis) versus B (y-axis) at day 0 (before activation of knock-down by doxycycline treatment). One dot represents one shRNA (total library 2,252 shRNA, 468 genes). *Mof* hairpins are highlighted in red. All hairpins with less than 600 reads were excluded from the analysis.

(B) Plotted is the Hiseq RPM for replicate A (x-axis) versus B (y-axis) at day 12 after knock-down. *Mof* hairpins are highlighted in red.

Valerio et al. Figure S2

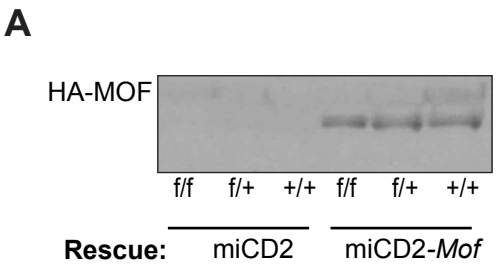



**Figure S2. *Mof* loss in a murine *MLL-AF9* leukemia model leads to impaired colony-forming capacity, a phenotype rescued by exogenous full-length *Mof*. Related to Figure 2.**

(A) *MA9 in vitro* transformed *Mof* $^{f/f}$, *Mof* $^{f/+}$ or *Mof* $^{+/+}$ LSKs were infected with HA-tagged full-length *Mof* or empty vector control (miCD2), and selected by sorting hCD2 positive cells. Western blot for HA confirmed the presence of the exogenous construct.

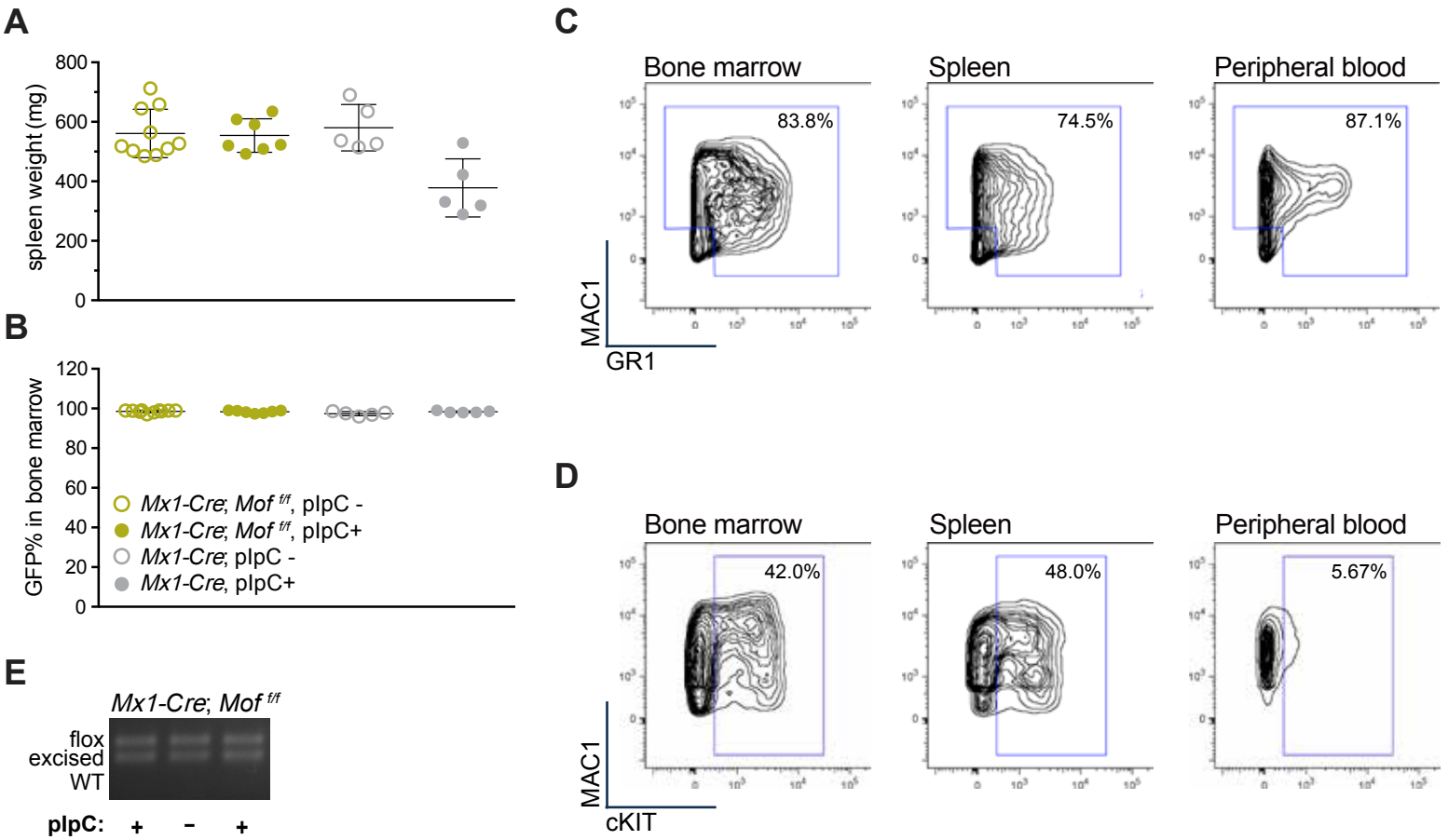



**Figure S3. *Mof* loss leads to reduced tumor burden and prolonged survival in an *in vivo MLL-AF9* leukemia model. Related to Figure 3.**

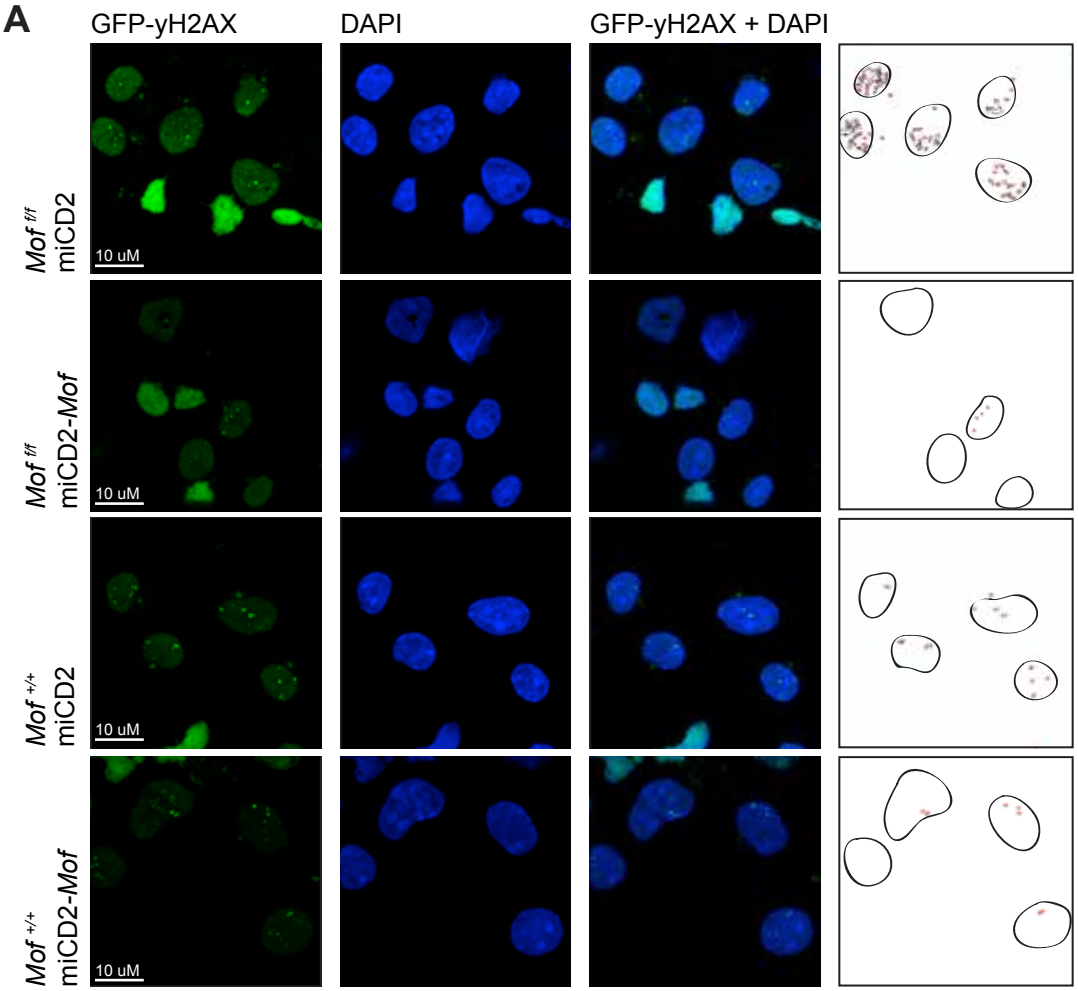
(A) Primary, GFP-tagged *MA9* leukemia BM cells in an *Mx1-Cre; Mof*$^{f/f}$ or *Mx1-Cre* background were injected into sub-lethally irradiated C57Bl/6 mice (n=10 for *Mx1-Cre* and n=20 for *Mx1-Cre; Mof*$^{f/f}$). Half of the mice per group were treated with poly-IC (pIpC) at day 15 post transplant to induce *Mof* excision. Plotted is the spleen weight of mice at time of death. A dot represents a single mouse in the experiment.

(B) Graph illustrating the GFP% of live cells in bone marrow of mice at time of death.

(C) FACS plots illustrating presence of myeloid cell surface markers (MAC1 and GR1) in GFP$^+$ bone marrow, spleen and peripheral blood cells at time of death. Representative images are shown.

(D) FACS plots illustrating presence of myeloid cell surface markers (MAC1 and cKIT) in GFP$^+$ bone marrow, spleen and peripheral blood cells at time of death. Representative images are shown.

(E) PCR analysis illustrating *Mof* excision at time of death. A representative gel image is shown.



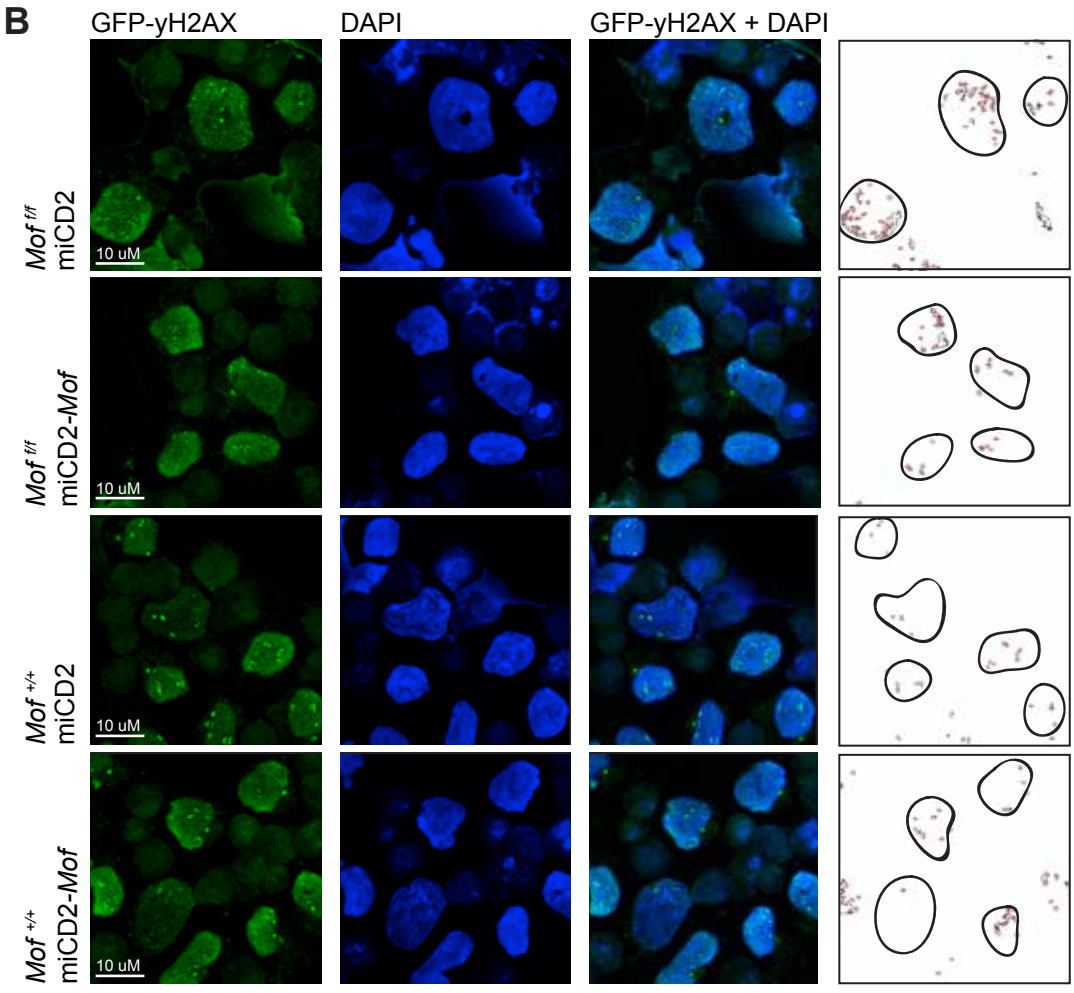




**Figure S4. yH2AX staining of *MLL-AF9* transformed cells. Related to Figure 4.**

(A) *MA9 in vitro* transformed *Mof*$^{f/f}$ or *Mof*$^{+/+}$ LSKs were infected with full-length *Mof* or empty vector control (miCD2) and selected by sorting hCD2 positive cells. Cells were fixed 48 hours after infection with dTomato-*Cre* and stained with a yH2AX antibody, a secondary GFP-labelled antibody and DAPI. The first three columns depict immunofluorescence images. The column on the right shows the outline of the counted cells and in red the foci as identified by ImageJ software. Representative images are shown.

(B) Same as A, but cells were cultured for another 24 hours before fixing and stainig so 72 hours after *Cre* infection.

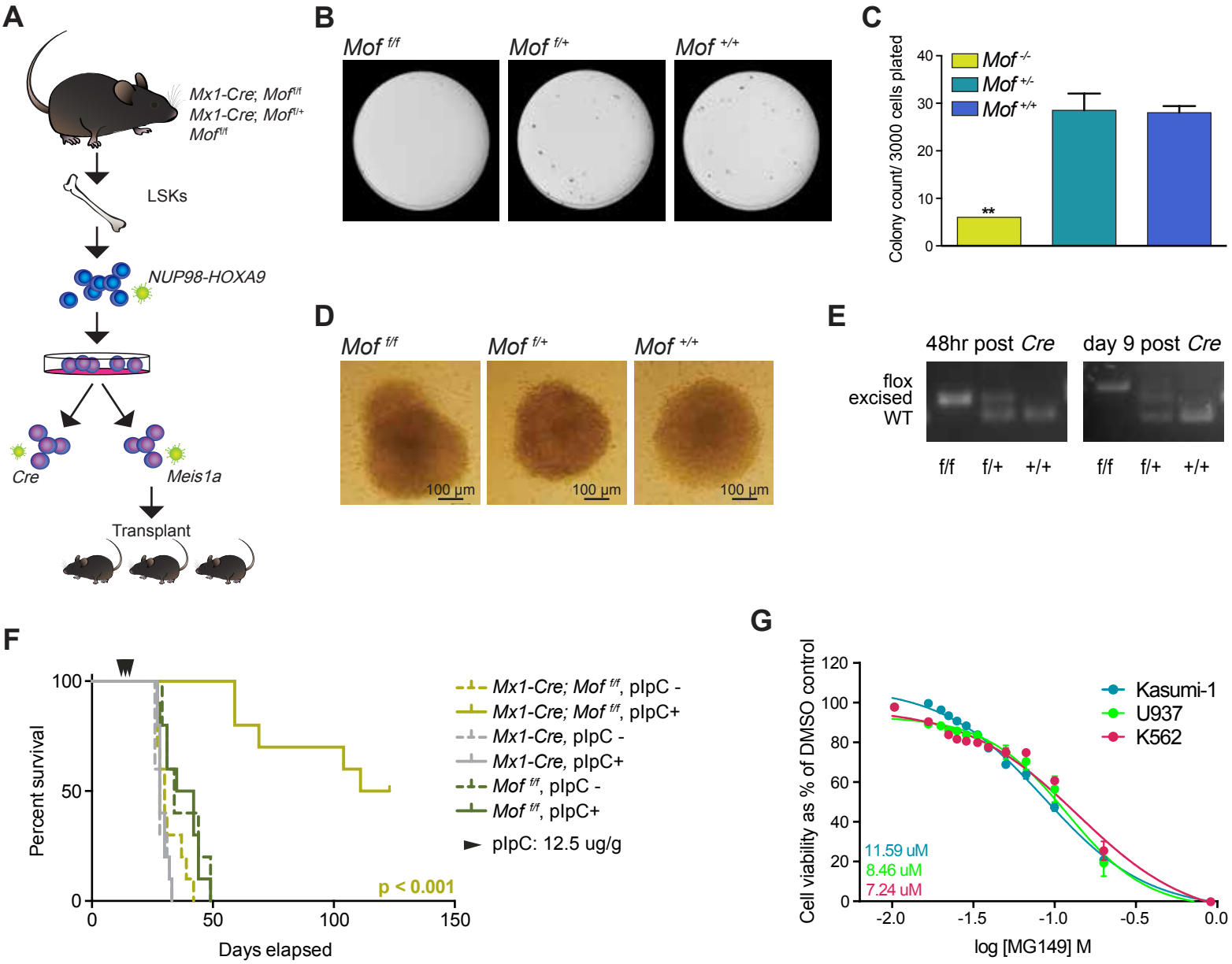



**Figure S5. *Mof* is required for various fusion-driven acute leukemias. Related to Figure 5.**

(A) Schematic for *in vitro* and *in vivo Mof* knockout experiments in a *NUP98-HOXA9* fusion-driven leukemia model.

(B) Day seven of methylcellulose colony-forming assay of *NUP98-HOXA9 in vitro* transformed *Mof*$^{f/f}$, *Mof*$^{f/+}$ or wild type (*Mof*$^{+/+}$) LSKs plated immediately upon sorting *Cre* positive cells. Representative petri dishes are shown.

(C) Bar graph indicating mean number of colonies per 35mm dish after seven days. 3000 cells were plated per dish. Data are representative of three individual experiments.

(D) Colonies at day seven of CFU assay. Representative images are shown.

(E) PCR analysis illustrating excision at indicated time points of the 7-day colony-forming experiment. Representative gel images are shown.

(F) 15,000 murine *NUP98-HOXA9*/*Meis1a* primary leukemia BM cells were injected into sub-lethally irradiated C57Bl/6 mice (n=20 per group). Half of the mice per group were treated with poly-IC (pIpC) at day 14 post transplant to induce *Mof* excision. Shown is the survival curve. Arrows indicate pIpC treatment. The p-value is calculated by log-rank test comparing survival of *Mx1-cre; Mof*$^{f/f}$ mice treated with pIpC to any of the control groups.

(G) Kasumi-1, U937 and K562 cells were plated in liquid culture and treated with various concentrations of MG149. Plotted is the IC50 curve for cell viability as a percentage of the vehicle (DMSO) control at day three of treatment. Numbers indicate the IC50 per cell type (by color).