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Leukemia and chromosomal instability in aged *Fancc−/−* mice

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Abstract

Fanconi anemia (FA) is an inherited disorder of genomic instability associated with high risk of myelodysplasia and AML. Young mice deficient in FA core complex genes do not naturally develop cancer, hampering preclinical studies on malignant hematopoiesis in FA. Here we show that aging *Fancc−/−* mice are prone to genomically unstable AML and other hematologic neoplasms. We demonstrate that aneuploidy precedes malignant transformation during *Fancc−/−* hematopoiesis. Our observations reveal that *Fancc−/−* mice develop hematopoietic chromosomal instability followed by leukemia in age-dependent manner, recapitulating the clinical phenotype of human Fanconi anemia and providing a proof of concept for future development of preclinical models of FA-associated leukemogenesis.

INTRODUCTION

The Fanconi anemia (FA) signaling network protects genomic integrity and prevents cancer by facilitating interphase DNA repair and orchestrating cell division[1-3]. Germline biallelic mutations of any FA genes cause Fanconi anemia (FA), an inherited bone marrow failure syndrome associated with myelodysplasia (MDS) and acute myeloid leukemia (AML). The overall risk of leukemia in FA is increased 600-fold[4].

Young mice deficient in core FA genes do not spontaneously recapitulate clinical hematopoietic manifestations of Fanconi anemia[5]. *Fancc−/−* mice demonstrate hypersensitivity to cross-linking agents[6], decreased hematopoietic stem cell repopulating ability[7,8], and hypersensitivity to interferon-gamma[8] reflecting disruption of the FA

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signaling network during hematopoiesis. However, young *Fancc−/−* mice do not develop spontaneous leukemia or bone marrow failure[6,8]. One observation study of a small *Fancc−/−* mouse cohort (n=8) did not detect decreased survival[9]. However, FA patients very rarely develop AML in their first year of life[10] and two soft-tissue tumors (adenocarcinoma and histiocytic sarcoma) had been reported in >13-month old *Fancc−/−* mice[11]. Thus, we hypothesized that aging *Fancc−/−* mice may be predisposed to hematopoietic malignancies. If the absolute time to the onset of leukemia is similar in FA humans and mice, a long-term observation of FA−/− mice may be crucial to detect cancer predisposition. To address this translationally relevant question, we asked whether *Fancc−/−* mice develop malignancies as they age.

RESULTS & DISCUSSION

We observed cohorts of *wt* (n=20) and *Fancc−/−* (n=18) mice for 24 months, and noticed decreased survival of *Fancc−/−* mice (p=0.01, **Fig. 1A**). Five *Fancc−/−* mice (27.8%) died between 8 and 24 months of age due to leukemia or lymphoma (**Fig. 1B-L**). Specifically, we diagnosed acute myeloid leukemia (AML) in two moribund *Fancc−/−* mice with peripheral blasts, predominance of Gr-1+ (Ly-6G) peripheral blood low-density mononuclear cells (LDMNCs), and myeloid infiltrates around the liver vessels (**Fig. 1C-E**). One *Fancc−/−* mouse developed lethal B-cell acute lymphoblastic leukemia (ALL), as evidenced by expansion of B220+ blasts that replaced >90% of bone marrow and infiltrated the liver (**Fig. 1F-H**). Additionally, two *Fancc−/−* mice died due to metastatic abdominal T-cell lymphoma manifested by massive mesenteric lymph node conglomerates (**Fig. 1I-J**) accompanied by Cd3+ liver infiltrates (**Fig. 1K-L**) in the absence of bone marrow or peripheral blood abnormalities. After 24 months of observation, all surviving *Fancc−/−* and *wt* mice were sacrificed and examined by necropsy. 4/13 (30.8%) 2-year old *Fancc−/−* animals had hematopoietic solid tumors and/or peripheral blasts, consistent with leukemia/lymphoma. Serial blood counts did not reveal progressive pancytopenia in aging *Fancc−/−* mice, suggesting that the development of leukemia may not be preceded by bone marrow failure in this animal model of FA. Together, 9/18 *Fancc−/−* mice developed hematopoietic malignancies by two years of age (including 5 animals that died prematurely due to disease), compared to 0/20 control *wt* mice (**Fig. 1B**, p=0.0003). Thus, aging *Fancc−/−* mice are prone to hematopoietic neoplasms, reflecting the age-dependent risk of leukemia in FA patients[4,10,12].

We next asked whether *Fancc−/−* AML can be propagated in *wt* mice via competitive stem cell transplantation. We mixed donor *Fancc−/−* Cd45.2+ LDMNCs isolated from a moribund AML *Fancc−/−* mouse (**Fig. 1C-E**) with *wt* Cd45.1+ competitor LDMNCs at a 1:1 ratio and transplanted the mixed cells into 3 lethally irradiated *wt* recipients. Three *wt* recipients of age-matched *wt* Cd45.2+ LDMNCs mixed with *wt* Cd45.1+ LDMNCs served as controls (**Fig. 2A**). By 50 days post-transplantation, all recipients of *Fancc−/−* LDMNCs had died of AML, while control recipients of *wt* LDMNCs remained healthy (**Fig. 2B**). The diagnosis of AML was confirmed in all recipients by flow cytometry, peripheral blood smears (**Fig. 2C**) and splenomegaly (p=0.0216, **Fig. 2D**). Peripheral blood flow cytometry demonstrated increased Cd45.2+ chimerism (p=0.0436) in recipients of leukemic *Fancc−/−* LDMNCs

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compared to controls at one month post-transplant (**Fig. 2E-F**), highlighting the malignant potential of leukemic *Fancc−/−* LDMNCs to outcompete *wt* hematopoietic cells in the host marrow.

The FA signaling network maintains genomic integrity during *Fancc−/−* hematopoiesis *in vivo*[13] and genomic instability promotes cancer[14]. Thus, we asked whether leukemic *Fancc −/−* mice displayed increased chromosomal instability and whether chromosomal instability precedes the onset of leukemia during *Fancc−/−* hematopoiesis. We compared karyotypes of LDMNCs isolated from leukemic *Fancc−/−* to age-matched *wt* and healthy *Fancc−/−* marrows. Bone marrow cells isolated from healthy *Fancc−/−* mice had higher incidence of aneuploidy and increased frequency of abnormal mitotic figures than *wt* LDMNCs (**Fig. 3A-D**), showing that *Fancc−/−* hematopoietic cells become chromosomally unstable before overt leukemogenesis occurs. Similarly, FA patients develop hematopoietic chromosomal and nuclear abnormalities prior to the onset of leukemia[13,15,16]. Leukemic *Fancc−/−* bone marrows were more aneuploid (**Fig. 3B**) with higher mitotic index compared to both age-matched *wt* ($p=0.001$) and *Fancc−/−* non-leukemic marrows ($p<0.0001$, **Fig. 3D**). This observation is consistent with further exacerbation of genomic instability and acquisition of bizarre karyotypic abnormalities reported in human FA-associated AML[17,18].

In summary, *Fancc−/−* mice develop chromosomally unstable hematopoietic malignancies as they age, recapitulating clinical and genomic abnormalities seen in Fanconi anemia patients (**Figs. 1** and **3**). Interestingly, similar incidence of tumors had been reported in old mice deficient in another FA core gene, *Fanca*, although that observation did not reach statistical significance due to small sample sizes[19]. Thus, late-onset carcinogenesis may be a common phenotype of murine FA core gene knockouts. AML arising in *Fancc−/−* mice can be propagated via hematopoietic stem cell transplant and produce rapid onset of lethal leukemia in *wt* transplant recipients (**Fig. 2**). As large-scale cohorts of leukemic mice are essential for preclinical drug testing, our observations may facilitate the development of future preclinical models of *FA−/−* AML.

METHODS

Mice

C57Bl/6J *Fancc−/−* mice were a gift of David W. Clapp (Indiana University). Mice were PCR-genotyped as described[9]. B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ mice were purchased from the IU In Vivo Therapeutics Core. All studies were approved by the Institutional Animal Care and Use Committee at IU.

Marrow harvest and transplantation

Bone marrow cells were flushed from mouse femurs using a 23-gauge needle/syringe (Becton Dickinson). LDMNCs were isolated by density gradient using Histopaque-1119 (Sigma) centrifuging for 30 minutes at 1800rpm with no brake. After centrifugation, LDMNCs were removed from the interface and utilized for experiments. Cytospins were made by resuspending LDMNCs in PBS and centrifuging onto slides at 450rpm for 5 minutes on a Shandon Cytospin 3 Cytocentrifuge (Thermo Scientific). 1.5×10^{6} donor test

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LDMNCs (C57Bl/6J background) and 1.5×10^6 donor competitor BoyJ LDMNCs were transplanted into recipients via tail vein injection. Recipients were 8 week-old female B6.SJL-*Ptprc*^{a}*Pepc*^{b}/BoyJ mice that underwent whole-body split-dose 1100 rads irradiation (700 rads/400 rads, 4 hours apart). For chimerism analysis, peripheral blood was collected from lateral tail veins into EDTA-coated tubes, incubated with RBC lysis solution (Qiagen) for 10 minutes at room temperature, washed, stained with anti-Cd45.2-FITC (BD Biosciences) and anti-Cd45.1-PE (BD Biosciences) as described[9], and analyzed on a FacsCalibur machine (Becton-Dickinson). At least 10,000 events/sample were acquired and analyzed using FlowJo Software.

Metaphase spreads

Bone marrow cells flushed from tibias were cultured in IMDM plus 20% FBS, murine SCF (100ng/ml), and IL-6 (200ng/ml) for 2 days. Cells were then exposed to 0.2 μg/ml colcemid (Life Tech) for 4 hours and pelleted at 800rpm for 5 minutes. Cells were resuspended dropwise in pre-warmed (37°C) 75mM KCl, and incubated at 37°C for 15 minutes. After pelleting, cells were resuspended in a 3:1 methanol:glacial acetic acid fixative. Cells were pelleted and resuspended in fixative two additional times before being dropped onto slides and dried overnight. Spreads were stained with Vectashield mounting medium with DAPI (Vector Laboratories).

Histology and flow cytometry

Murine tissues obtained post-mortem were fixed in 10% formalin, paraffin-embedded, sectioned (5 μM sections), and stained with hematoxylin and eosin. Peripheral blood smears and bone marrow cytospins were stained with Giemsa using the automated Siemens Hematek 3000 (Fisher) system. For flow cytometry, peripheral blood cells were incubated in RBC lysis solution and bone marrow LDMNCS were isolated as described above. Cells were stained with either Gr-1-APC (Ly6G, clone: RB6-8C5) or B220-FITC (clone: RA3-6B2), analyzed on a FacsCalibur machine using live gating followed by data quantification with FlowJo software. Leukemia diagnoses were made using criteria established in the Bethesda proposal for classification of nonlymphoid neoplasms in mice[20], and were independently validated by a veterinary pathologist at IU School of Medicine.

Microscopy

Images of smears, cytospins, and histological sections were obtained using a Zeiss Axiolab microscope with a color camera. Metaphase spreads were imaged on a Deltavision personalDx deconvolution microscope (Applied Precision). Image stacks (distance between z-sections: 0.2 μm) were deconvolved using Softworx and analyzed using Imaris software suite (Bitplane).

Statistics

Statistical analysis was performed with the GraphPad Prism 6 software.

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REFERENCES

1. Kottemann MC, Smogorzewska A. Fanconi anaemia and the repair of Watson and Crick DNA crosslinks. Nature. 2013; 493(7432):356–363. [PubMed: 23325218]
2. Nalepa G, Clapp DW. Fanconi anemia and the cell cycle: new perspectives on aneuploidy. F1000Prime Rep. 2014; 6:23. [PubMed: 24765528]
3. D'Andrea AD. Susceptibility pathways in Fanconi's anemia and breast cancer. N Engl J Med. 2010; 362(20):1909–1919. [PubMed: 20484397]
4. Alter BP. Fanconi anemia and the development of leukemia. Best Pract Res Clin Haematol. 2014; 27(3-4):214–221. [PubMed: 25455269]
5. Parmar K, D'Andrea A, Niedernhofer LJ. Mouse models of Fanconi anemia. Mutat Res. 2009; 668(1-2):133–140. [PubMed: 19427003]
6. Chen M, Tomkins DJ, Auerbach W, et al. Inactivation of Fac in mice produces inducible chromosomal instability and reduced fertility reminiscent of Fanconi anaemia. Nat Genet. 1996; 12(4):448–451. [PubMed: 8630504]
7. Haneline LS, Gobbett TA, Ramani R, et al. Loss of FancC function results in decreased hematopoietic stem cell repopulating ability. Blood. 1999; 94(1):1–8. [PubMed: 10381491]
8. Whitney MA, Royle G, Low MJ, et al. Germ cell defects and hematopoietic hypersensitivity to gamma-interferon in mice with a targeted disruption of the Fanconi anemia C gene. Blood. 1996; 88(1):49–58. [PubMed: 8704201]
9. Pulliam-Leath AC, Ciccone SL, Nalepa G, et al. Genetic disruption of both Fancc and Fancg in mice recapitulates the hematopoietic manifestations of Fanconi anemia. Blood. 2010; 116(16):2915–2920. [PubMed: 20606166]
10. Alter BP, Giri N, Savage SA, et al. Malignancies and survival patterns in the National Cancer Institute inherited bone marrow failure syndromes cohort study. Br J Haematol. 2010; 150(2):179–188. [PubMed: 20507306]
11. Carreau M. Not-so-novel phenotypes in the Fanconi anemia group D2 mouse model. Blood. 2004; 103(6):2430. [PubMed: 14998919]
12. Kutler DI, Singh B, Satagopan J, et al. A 20-year perspective on the International Fanconi Anemia Registry (IFAR). Blood. 2003; 101(4):1249–1256. [PubMed: 12393516]
13. Abdul-Sater Z, Cerabona D, Sierra Potchanant E, et al. FANCA safeguards interphase and mitosis during hematopoiesis in vivo. Exp Hematol. 2015
14. Gordon DJ, Resio B, Pellman D. Causes and consequences of aneuploidy in cancer. Nat Rev Genet. 2012; 13(3):189–203. [PubMed: 22269907]
15. Mehta PA, Harris RE, Davies SM, et al. Numerical chromosomal changes and risk of development of myelodysplastic syndrome--acute myeloid leukemia in patients with Fanconi anemia. Cancer Genet Cytogenet. 2010; 203(2):180–186. [PubMed: 21156231]
16. Barton JC, Parmley RT, Carroll AJ, et al. Preleukemia in Fanconi's anemia: hematopoietic cell multinuclearity, membrane duplication, and dysgranulogenesis. J Submicrosc Cytol. 1987; 19(2): 355–364. [PubMed: 3599130]
17. Woo HI, Kim HJ, Lee SH, Yoo KH, Koo HH, Kim SH. Acute myeloid leukemia with complex hypodiploidy and loss of heterozygosity of 17p in a boy with Fanconi anemia. Ann Clin Lab Sci. 2011; 41(1):66–70. [PubMed: 21325258]

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18. Quentin S, Cuccuini W, Ceccaldi R, et al. Myelodysplasia and leukemia of Fanconi anemia are associated with a specific pattern of genomic abnormalities that includes cryptic RUNX1/AML1 lesions. Blood. 2011; 117(15):e161–170. [PubMed: 21325596]

19. Wong JC, Alon N, McKerlie C, Huang JR, Meyn MS, Buchwald M. Targeted disruption of exons 1 to 6 of the Fanconi Anemia group A gene leads to growth retardation, strain-specific microphthalmia, meiotic defects and primordial germ cell hypoplasia. Hum Mol Genet. 2003; 12(16):2063–2076. [PubMed: 12913077]

20. Kogan SC, Ward JM, Anver MR, et al. Bethesda proposals for classification of nonlymphoid hematopoietic neoplasms in mice. Blood. 2002; 100(1):238–245. [PubMed: 12070033]

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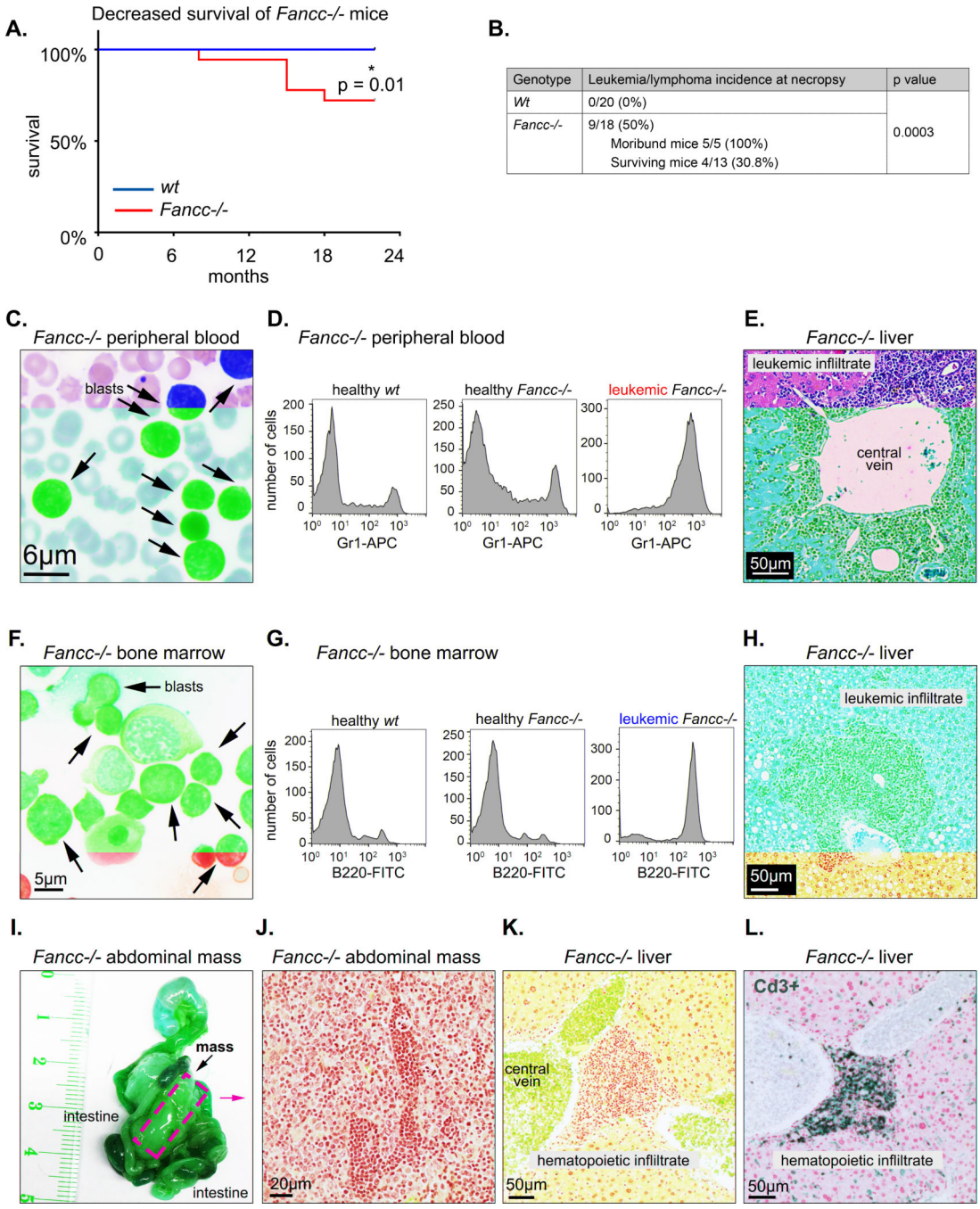

Genotype	Leukemia/lymphoma incidence at necropsy	p value
Wt	0/20 (0%)	0.0003
Fancc-/-	9/18 (50%) Moribund mice 5/5 (100%) Surviving mice 4/13 (30.8%)	

Fig 1. Aging *Fancc−/−* mice develop hematologic malignancies

(**A**) Kaplan-Meier survival curve of *wt* (n=20) and *Fancc−/−* (n=18) cohorts. (**B**) Table demonstrating incidence of leukemias and lymphomas in *wt* and *Fancc−/−* mice by 24 months of age. Statistical significance for (**A, B**) was determined using a log-rank (Mantel-Cox) test. Peripheral blood smear of a moribund *Fancc−/−* mouse (**C**) shows leukemic blasts (arrows). Diagnosis of acute myeloid leukemia was confirmed with flow cytometry demonstrating increased expression of the Gr1 myeloid marker in the peripheral blood compared to *wt* and healthy *Fancc−/−* controls (**D**) and the presence of leukemic infiltrates

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in the liver (**E**). Bone marrow cytospin (**F**) of another moribund *Fancc−/−* mouse demonstrated multiple blasts (arrows). Flow cytometry demonstrated increased expression of the B220 B-cell marker on bone marrow blasts (**G**) and necropsy revealed leukemic infiltrates in the liver (**H**), consistent with B-cell ALL. Necropsy of another *Fancc−/−* mouse demonstrated conglomerates of mesenteric lymph nodes (**I, J**). Liver infiltrates in this mouse were Cd3+ (**K, L**), consistent with T-cell lymphoma.

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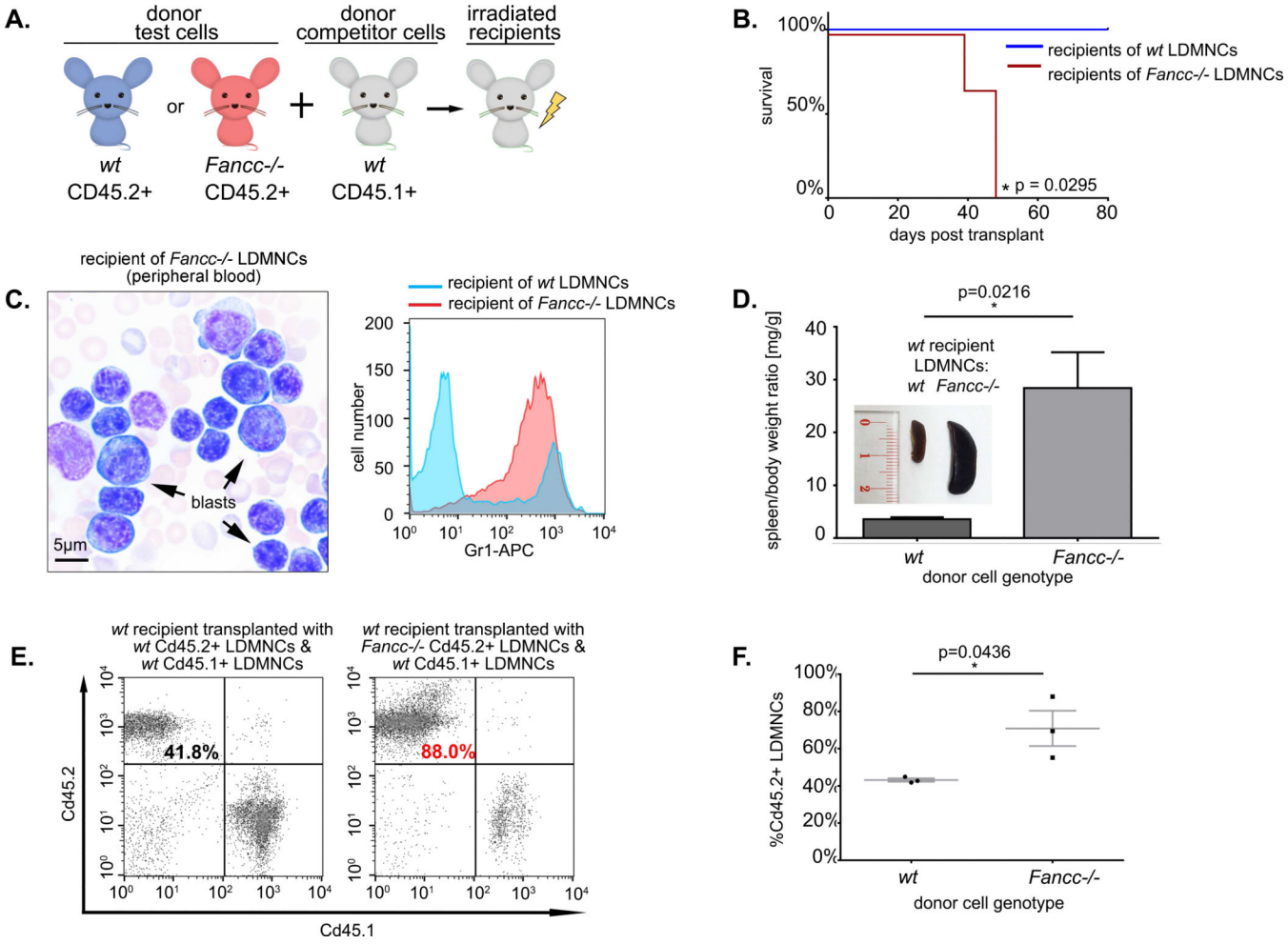

Fig 2. Transplanted *Fancc−/−* AML LDMNCs induce aggressive leukemia in *wt* recipients

(**A**) Competitive repopulation transplant schematic. Cd45.2+ LDMNCs obtained from a leukemic *Fancc−/−* mouse or *wt* control were mixed 1:1 with Cd45.1+ *wt* competitor LDMNCs and transplanted into lethally irradiated *wt* recipients (n=3 recipients per genotype). (**B**) Recipients of *Fancc−/−* LDMNCs died within 2 months after transplantation. Log-rank (Mantel-Cox) test was used to assess significance. (**C**) AML in *wt* recipients of *Fancc−/−* LDMNCs. Gr1 was used as a myeloid marker for flow cytometry (right panel). (**D**) Splenomegaly in moribund *wt* mice transplanted with *Fancc−/−* LDMNCs. (**E, F**) Leukemic Cd45.2+ *Fancc−/−* LDMNCs overpopulate recipient bone marrow compared to *wt* Cd45.1+ competitor LDMNCs. Statistical significance was determined using an unpaired student's t-test; error bars represent SEM.

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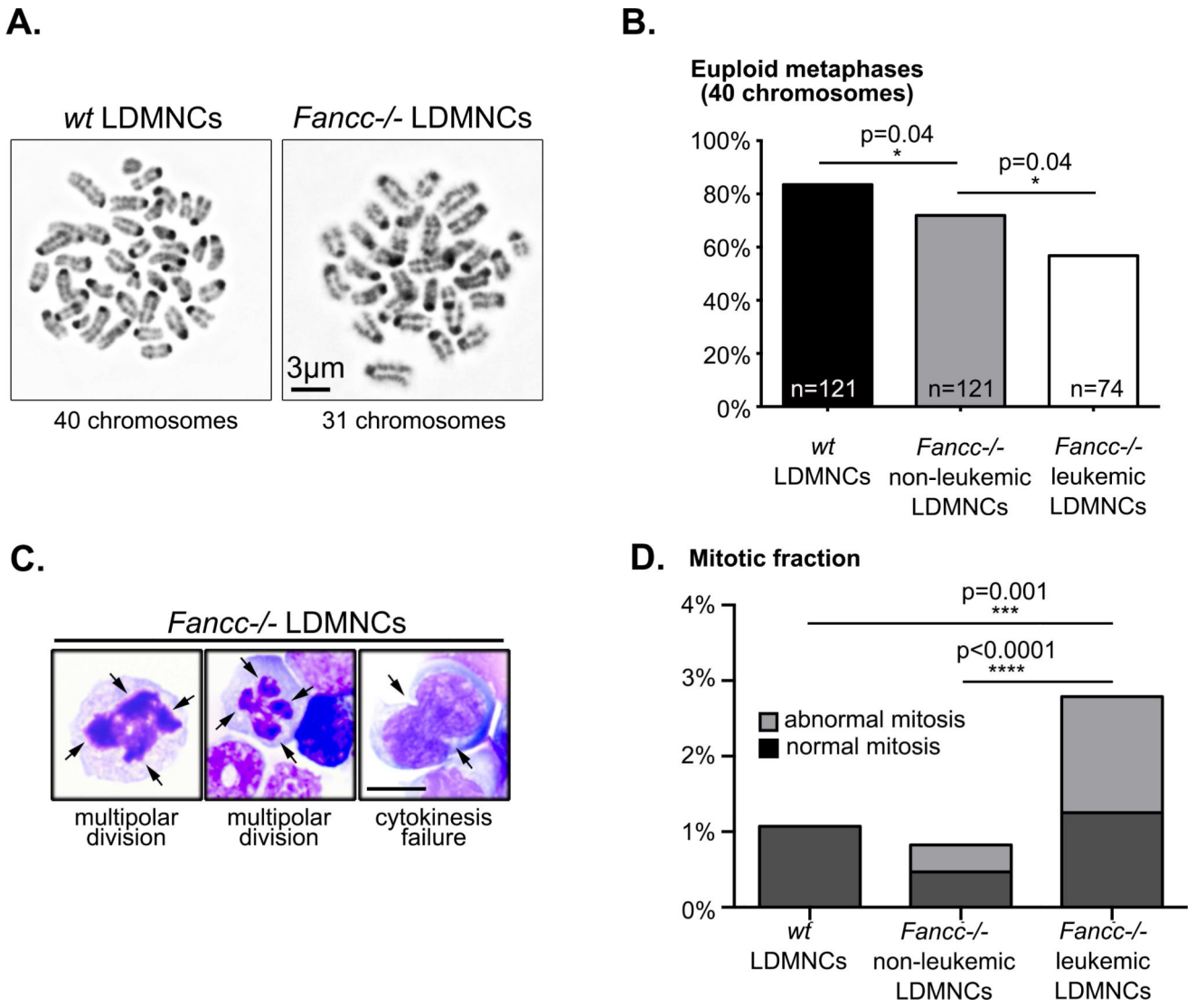

Fig 3. Genomic instability and abnormal mitosis in leukemic and pre-leukemic *Fancc−/−* mice

(**A**) Representative images of LDMNC metaphase spreads from *wt* and leukemic *Fancc−/−* mice. (**B**) Increased aneuploidy in leukemic *Fancc−/−* LDMNCs. At least 74 spreads were counted from *wt, Fancc−/−* non-leukemic, and *Fancc−/−* leukemic mice. Note increased chromosomal instability in non-leukemic *Fancc−/−* LDMNCs compared to age-matched *wt* controls. Fisher's exact test was used to determine statistical significance. Leukemic *Fancc−/−* LDMNCs undergo abnormal mitosis (**C**) and have a higher mitotic index (**D**) compared to LDMNCs from *wt* and *Fancc−/−* non-leukemic mice (n=3 mice/genotype; at least 500 cells were counted per genotype). Statistical analyses were performed using chi-square tests with Yates correction.