Cdx4 is dispensable for murine adult hematopoietic stem cells but promotes MLL-AF9-mediated leukemogenesis

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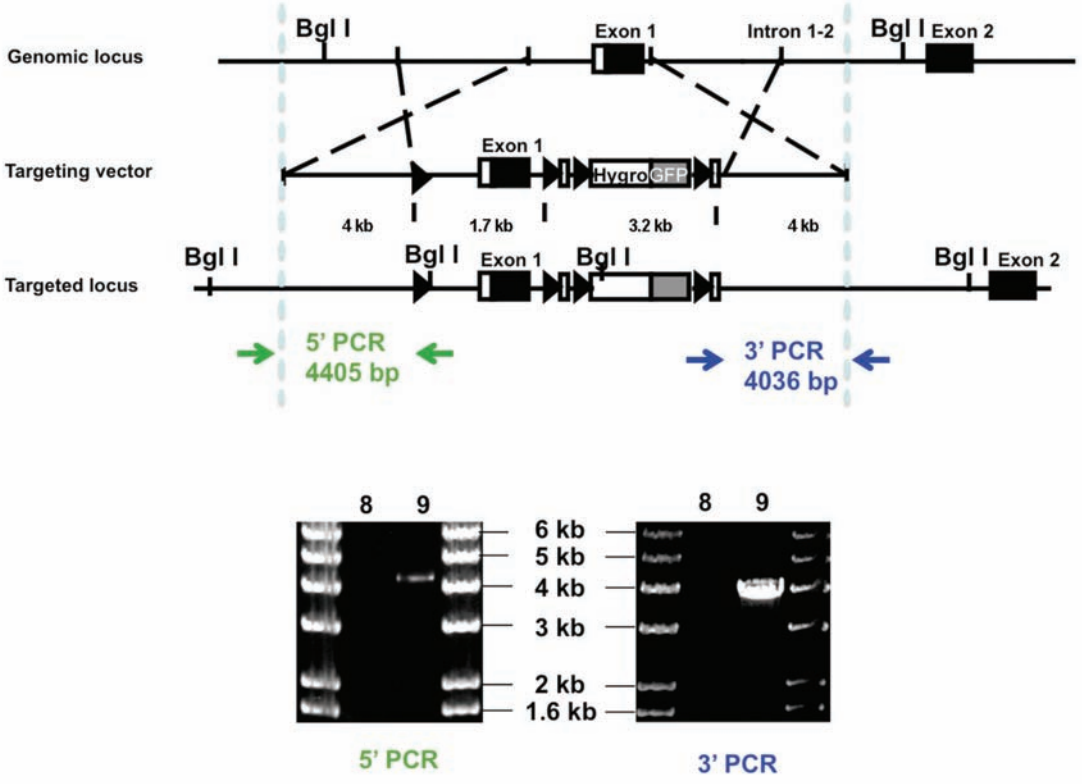
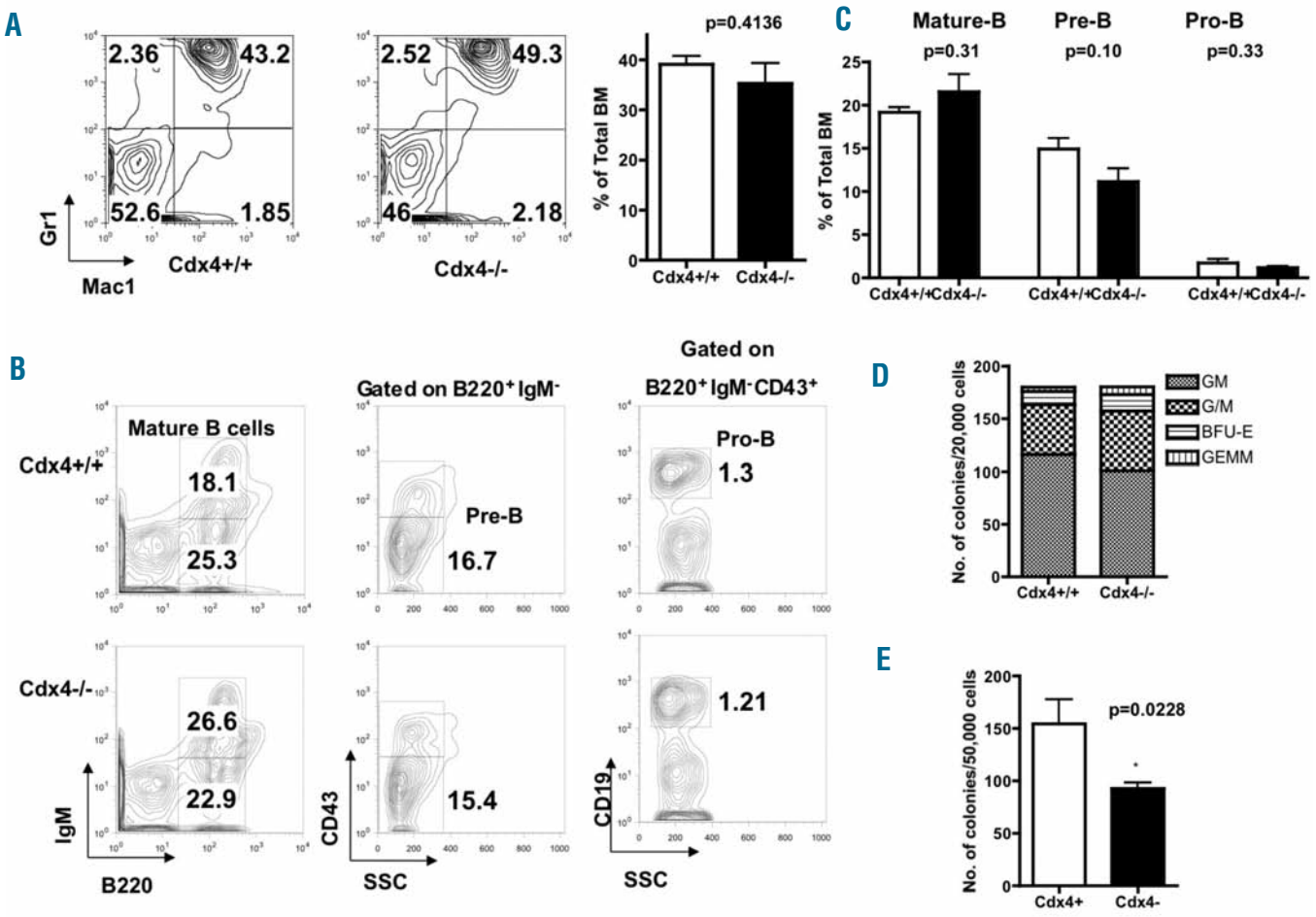
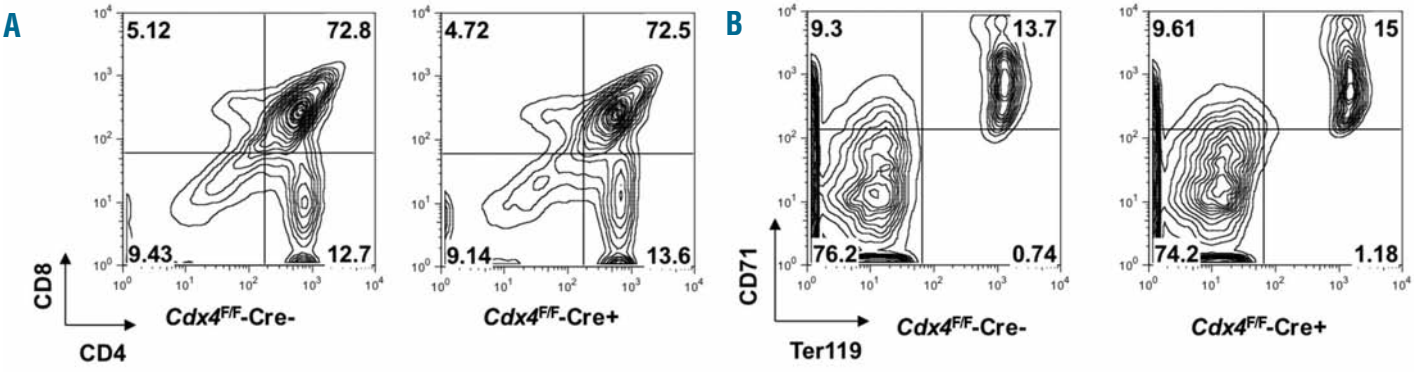


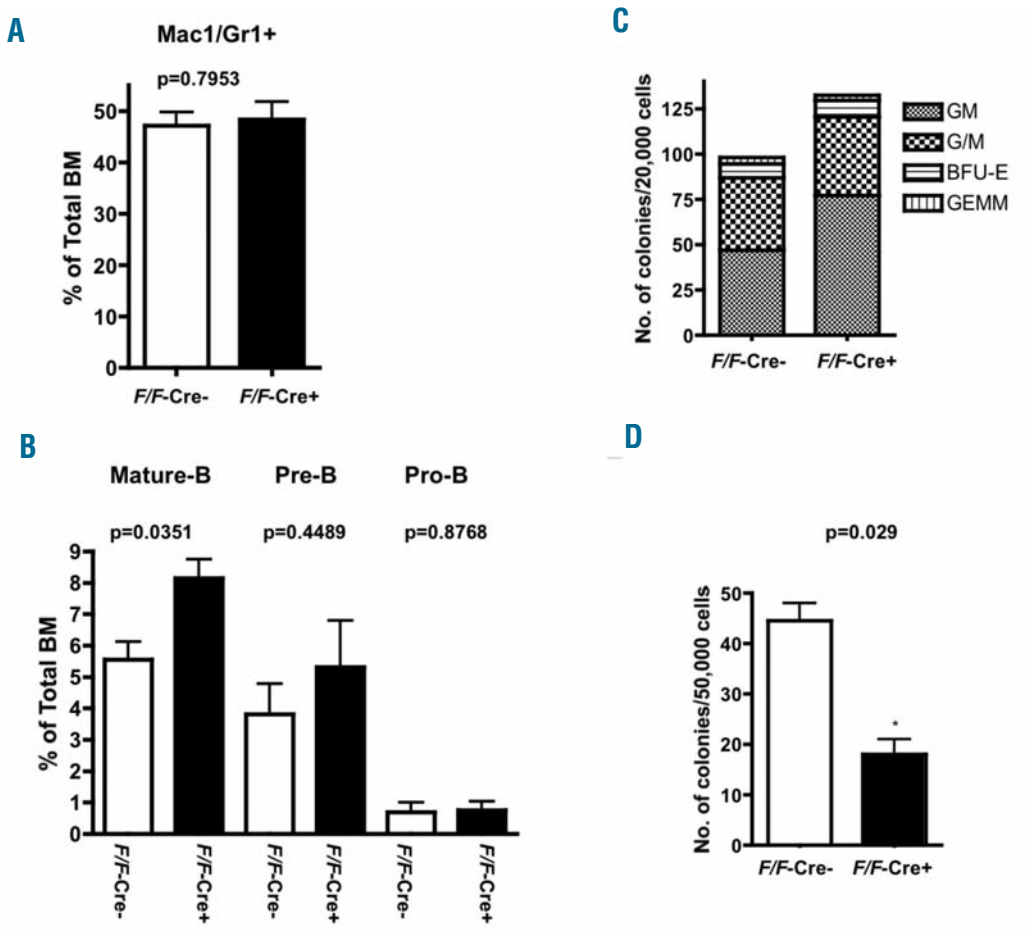
Online Supplementary Figure S1. Homologous recombination at the *Cdx4* locus. Primer pairs used to screen for homologous recombination of the targeting DNA on both sides of the construct are shown. Primer sequences are as follows: 5'PCR Forward: 5'-tttggggtcaggttctcatgt-3'; 5'PCR Reverse: 5'-tccggcataacttcgtatagca-3'; 3'PCR Forward: 5'- agagaataggaacttcggaatagaa-3'; 3'PCR Reverse: 5'-gattgtggtccttcccaacc-3'; Bottom panels: Clone 9 was correctly targeted while Clone 8 was not. Clone 9 was used to generate chimeras and obtain germline transmission of the *Cdx4*^{F} allele.



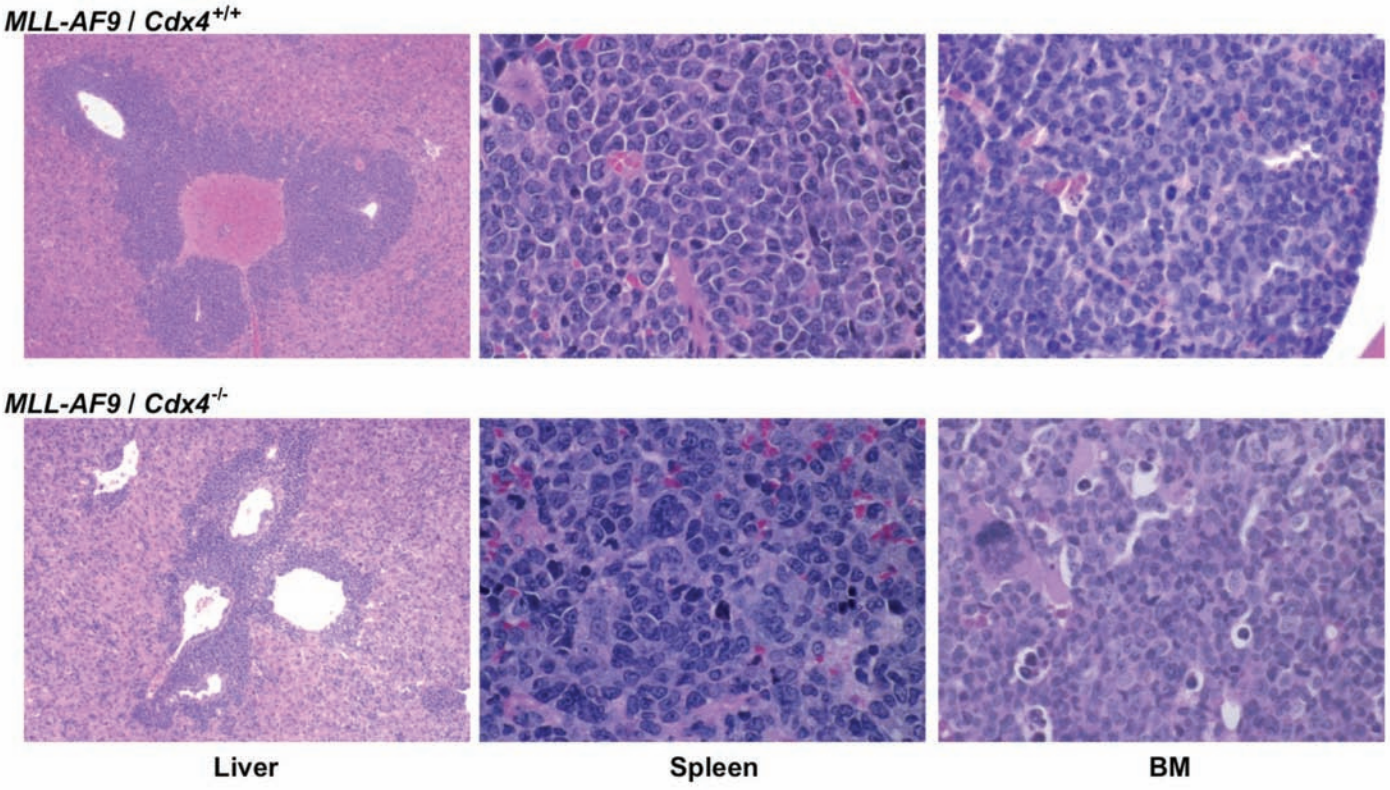
Online Supplementary Figure S2. Analysis of the hematopoietic compartment in *Cdx4*$^{-/-}$ mice. (A) A representative flow cytometric analysis shows comparable percentages of myeloid cells (Mac1^{+}Gr1^{+}) in *Cdx4*$^{-/-}$ and wild-type littermate controls (n=12). (B) A representative flow cytometric analysis of the B-cell lineage shows no significant differences in percentages of mature B cells, pre-B and pro-B cells in *Cdx4*$^{-/-}$ and wildtype littermate controls. (C) Histogram representation of results presented in (B) (n=4). (D) Plating of 20,000 bone marrow cells in M3434 medium (n=6). (E) Plating of 50,000 bone marrow cells in M3630 medium (n=4). Colonies were counted 10 days after plating. A decrease in pre-B colony number was seen in *Cdx4*$^{-/-}$ mice compared to wild-type littermate controls.



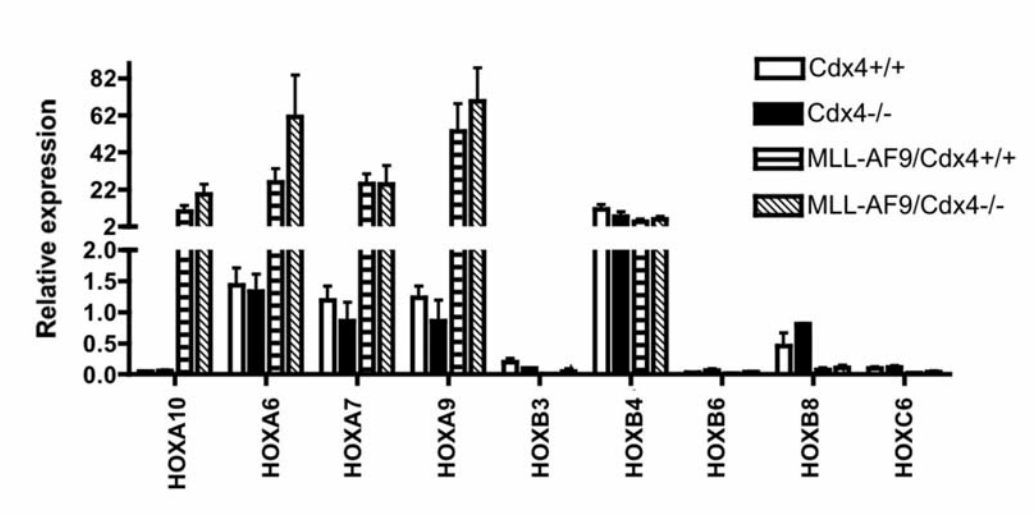
Online Supplementary Figure S3. Analysis of the T-cell and erythroid compartments. Flow cytometric analysis shows comparable percentages of (A) CD4 and CD8 marker expression in the thymus and (B) erythroid markers in bone marrow cells between *Cdx4*$^{F/F}$- *Cre*$^{+}$ and control littermates.



Online Supplementary Figure S4. Analysis of the hematopoietic compartment in older *Cdx4*$^{F/F}$-*Cre*$^{+}$ mice Analysis was performed 8-12 months after pIpC treatment of *Cdx4*$^{F/F}$-*Cre*$^{+}$ mice. (A) Flow cytometric analysis shows comparable percentages of myeloid cells (Mac1^{+}Gr1^{+}) and (B) various B-cell populations in older *Cdx4*$^{F/F}$-*Cre*$^{+}$ and control mice. (C) Plating of 20,000 bone marrow cells in M3434 medium (n=4). No difference was seen in older *Cdx4*$^{F/F}$-*Cre*$^{+}$ mice and wild-type littermates (D) Plating of 50,000 bone marrow cells in M3630 medium (n=4). A decrease in pre-B colony number was seen in older *Cdx4*$^{F/F}$-*Cre*$^{+}$ mice.



Online Supplementary Figure S5. Analysis of *MLL*-AF9-mediated transformation in a *Cdx4*$^{-/-}$ background. Histo-pathological analysis of tissues from mice transplanted with MLL-AF9-expressing wild-type or *Cdx4*$^{-/-}$ bone marrow cells. Original magnifications x100 (liver) and x1000 (spleen and bone marrow: BM).



Online Supplementary Figure S6. *Hox* gene expression patterns in *Cdx4*-deficient mice. Histogram representation of the expression of a selected set of Hox genes relative to β-*actin*. Hox expression patterns between *Cdx4*$^{-/-}$ and *Cdx4*$^{+/+}$ control mice were compared under steady-state conditions (at 8 weeks) and in *MLL-AF9*-transformed recipients. Values are represented as the mean ±SD of two independent experiments performed for each sample.

Online Supplementary Table S1. Peripheral blood cell counts of adult *Cdx4*$^{-/-}$ and wild-type mice. Blood samples were taken at 12 weeks, and complete blood counts were obtained with a Hemavet950 cell counter.

	Cdx4$^{+/+}$ (n=12)	*Cdx4*$^{-/-}$ (n=12)	t-test (p-value)
WBC (x10^9/L)	3.933 ± 0.3005	7.447 ± 1.904	0.012
Lymphocytes (x10^9/L)	2.767 ± 0.3398	4.297 ± 1.742	0.0609
Granulocytes (x10^9/L)	0.7800 ± 0.2228	2.553 ± 0.7074	0.0020
Monocytes (x10^9/L)	0.2356 ± 0.0403	0.3089 ± 0.0683	0.0470
Red blood cell(x10^{12}/L)	8.211 ± 0.6290	9.026 ± 0.4510	0.0274
Hematocrit (%)	43.66 ± 3.824	50.08 ± 3.116	0.0097
Platelets (x10^9/L)	554.1 ± 81.28	673.3 ± 141.8	0.1043

WBC: white blood cells

Online Supplementary Table S2. Analysis of hematopoietic tissues in *Cdx4*$^{-/-}$ and *Cdx*$^{F/F}$-*Cre*$^{+}$ mice. Animals were analyzed at 12 weeks (Cdx4$^{-/-}$ mice) or 6-8 weeks post-pIpC treatment (*Cdx4*$^{F/F}$ mice). Spleen and liver weights were obtained, and bone marrow nucleated cells were counted after lysis of red blood cells.

	Cdx4$^{+/+}$	*Cdx4*$^{-/-}$	t-test (p-value)
Spleen weight (mg)	75.33 ± 4.768	74.67 ± 5.152	0.8225
Liver weight (g)	1.156 ± 0.070	1.223 ± 0.124	0.2759
Bone marrow cell counts (x10^6)	38.92 ± 2.478	34.15 ± 1.572	0.0481

	Cdx4$^{F/F}$-Cre-	*Cdx4*$^{F/F}$-Cre+	t-test (p-value)
Spleen weight (mg)	83.33 ± 9.545	88.33 ± 8.724	0.3659
Liver weight (g)	1.521 ± 0.123	1.536 ± 0.141	0.8483
Bone marrow cell counts (x10^6)	33.96 ± 1.160	37.28 ± 3.858	0.0711