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Figure legends

Fig. 1. IDH2/R140Q and NPMc upregulate *Meis1* and *Hoxa9*, respectively.

A) Schema of the experiments. c-Kit$^+$ hematopoietic progenitor cells were isolated from mouse bone marrow (BM) and infected with pMy-NPMc-ires-EGFP, pMy-DNMT3A/R882H-ires-EGFP, pGCDN-IDH2/R140Q-ires-NGFR, or pGCDN-FLT3/ITD-ires-NGFR. After incubation in liquid culture, EGFP$^+$ or NGFR$^+$ cells were sorted by flow cytometry and cultured in methylcellulose medium.

B) IDH2/R140Q and NPMc induced expression of *Meis1* and *Hoxa9*, respectively. RNA was prepared from first-colony samples and analyzed by quantitative RT-PCR.

C) NPMc confers serial colony-forming activity on *Npm1*$^{+/-}$ cells but not on wild-type (WT) cells. c-Kit$^+$ hematopoietic progenitor cells from WT and *Npm1*$^{+/-}$ mouse BM were infected with NPMc-ires-EGFP, and EGFP$^+$ cells were cultured in methylcellulose medium.

D) *Hoxa9* mRNA levels induced by NPMc are higher in the *Npm1*$^{+/-}$ than in WT cells. *P < 0.1, **P < 0.05, and ***P < 0.001.

Fig. 2. Establishment of an AML model harboring IDH2/R140Q.

A) Scheme of the experiments. c-Kit$^+$ hematopoietic progenitor cells were isolated from *Npm1*$^{+/-}$ mouse bone marrow (BM) and serially infected with three or four of the following constructs: pMy-NPMc-ires-EGFP, pGCDN-IDH2/R140Q-ires-NGFR, pMSCV-DNMT3A/R882H-puro, and pMSCV-FLT3/ITD-neo. When cells were infected with three mutant genes, the infection of corresponding empty vector for the absent mutant gene was performed. The infectants were transplanted into irradiated mice.

B) NPMc, IDH2/R140Q, DNMT3A/R882H, and FLT3/ITD (NIDF) cooperatively induce AML in mice. The abbreviations for the gene combinations (IDF, NDF, NIF, NID, and NIDF) are indicated. IDF vs. NIDF, P < 0.0001; NDF vs. NIDF, P < 0.0001; NIF vs. NIDF, P = 0.0009; NID vs. NIDF, P = 0.0006; log-rank test.

C) Schema of pMy-NPMc-ires-EGFP and pGCDN-IDH2/R140Q-ires-NGFR vectors.

D) Flow cytometric analysis of EGFP and NGFR in BM cells from AML mice

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transplanted with NIDF cells. The bar graph represents the mean percentage of $NPMc^+IDH2/R140Q^+$ cells in the BM for 4 mice studied.

E) Morphology of BM cells isolated from NIDF-induced AML mice. The bar graph shows the percentage of blast cells in BM samples from moribund mice transplanted with NIDF, NID, and NIF cells.

F) Flow cytometric analysis of AML cells. BM cells from NIDF-induced AML mice were analyzed for Mac1 and Gr1 expression. The bar graph represents the mean percentage of $Mac1^+Gr1^+$ fraction in AML cells for 4 mice studied.

G) BM cells (1×10^5 or 1×10^6) from NIDF-induced AML mice were transplanted into the irradiated mice. All secondary recipient mice died of AML.

Fig. 3. IDH2/R140Q is necessary for the maintenance of AML.

A) Schematic of the IDH2/R140Q-flox system. Because loxP sequences were inserted in both 5' and 3' regions of *IDH2/R140Q* on the vector, *IDH2/R140Q* can be deleted by activating Cre recombinase.

B) Experimental scheme. c-Kit^+ hematopoietic progenitor cells were isolated from ERT2-Cre^+ *Npm1*$^{+/-}$ mice, serially infected with pMy-NPMc-ires-EGFP, pGCDN-flox-IDH2/R140Q-ires-NGFR, pMSCV-DNMT3A/R882H-puro, and pMSCV-FLT3/ITD-neo [Nf(I)DF], and then transplanted into irradiated mice. The Nf(I)DF-induced AML cells were transplanted into secondary recipient mice. After 7 weeks (C, D), 3 weeks (E), and 2 weeks (F, G), the secondary recipient mice were injected with corn oil or tamoxifen (TAM).

C) The genotyping of floxed *IDH2/R140Q*. Genomic DNA was prepared from PB cells 0, 1, 2, 4, and 7 days after TAM treatment, and genotyping of floxed and Δ *IDH2/R140Q* was performed.

D) 2-HG levels in blood plasma and peripheral blood (PB) cells. Plasma and PB cells were isolated from three mice described in (C).

E-F) Survival of secondary recipient mice treated with corn oil or TAM. TAM treatment began 3 weeks (E) or 2 weeks (F) after transplantation. The inset panels show the percentage of $EGFP^+$ leukemic cells in the PB at the start of tamoxifen treatment. E), $P = 0.0029$; F), $P < 0.0001$; log-rank test.

G) Deletion of IDH2/R140Q suppressed the expansion of $EGFP^+$ cells in PB. PB cells were isolated from the mice shown in Figure 3F 4 weeks after TAM treatment and

analyzed for NPMc (EGFP) and IDH2/R140Q (NGFR) expression by flow cytometry. The number represents the mean percentage of $EGFP^+$ cells in the PB of 10 mice.

H) Deletion of IDH2/R140Q decreased the population of $EGFP^+$ cells in PB. TAM treatment began 7 weeks after transplantation. PB cells were isolated from mice before and after TAM treatment (1, 2, and 4 weeks), and were analyzed by flow cytometry. The graph represents the mean percentage of $EGFP^+$ cells in the PB.

I) Deletion of IDH2/R140Q decreased the fraction of cells expressing LSC markers and exhausted $EGFP^+$ cells in BM. TAM treatment began 7 weeks after transplantation. BM cells were isolated from the mice shown in Figure 3H and analyzed for NPMc, EGFP, and NGFR by flow cytometry. The mean percentages of $EGFP^+$ cells in the BM of three mice are shown. Bar graphs show the populations of cells expressing several LSC markers (MCSFR, L-GMP (Lin^-, $Sca1^-$, $cKit^+$, $CD16/32^+$, $CD34^+$), $cKit^+Gr1^-$, and $CD34^+$) among $EGFP^+$ cells at 2 weeks after TAM treatment. Genotyping of IDH2/R140Q was performed with BM cells at 2 weeks after TAM treatment.

J) Deletion of IDH2/R140Q decreased the LSC population. BM cells treated or untreated with TAM for 2 weeks were isolated from the mice shown in Figure 3I. The third BMT was performed using these BM cells. The mice were not treated with tamoxifen after the third BMT. PB cells were isolated from mice 4 weeks after the third BMT and were analyzed for NPMc (EGFP) and IDH2/R140Q (NGFR) expression by flow cytometry. The number represents the mean percentage of $EGFP^+$ cells in the PB of 5 mice.

K) Survival curb of four mice after the third BMT.

L) Results of GSEA. The IDs of the gene sets in the MSigDB (Molecular Signature Database) are indicated. $*P < 0.1$, $**P < 0.05$.

Fig. 4. Functional analysis of four genes involved in AML development.

A) Flow cytometric analysis of EGFP and NGFR in bone marrow (BM) and peripheral blood (PB) cells from mice transplanted with NIDF cells 3 months after transplantation.

B) IDH2/R140Q is required for engraftment and survival of $NPMc^+$ cells *in vivo*. $c\text{-}Kit^+$ hematopoietic progenitor cells from *Npm1*$^{+/-}$ mice were infected with

viruses as indicated and transplanted into irradiated mice. PB cells were analyzed for expression of EGFP (NPMc) and NGFR (IDH2/R140Q) 8 weeks after transplantation. The number represents the mean percentage of $EGFP^+$ cells in the PB of 4 mice.

C) Representative FACS plots of NGFR in BM cells of mice at 6 months after transplantation of IDH2/R140Q-transduced cells. The bar graph shows the mean percentages of $IDH2/R140Q^+$ cell for all five mice studied.

D) Results of GSEA. The microarray data of RNA isolated from IDH2/R140Q expressing cells in Figure 4C and control BM cells were compared. The IDs of the gene sets in the MSigDB (Molecular Signature Database) are indicated.

E) Representative FACS plots of EGFP and NGFR in BM cells of mice at 6 month after transplantation of NI cells. The bar graph shows the mean percentages of $NPMc^+IDH2/R140Q^+$ cells for all four mice studied.

F) Representative morphology and blast population of NI cells. BM cells were prepared from the mice used in Figure 4E and stained with May–Giemsa. The bar graph shows the percentage of blast cells.

G) $IDH2/R140Q^+$ and $NPMc^+$ cells were enriched in BM. c-Kit^+ cells from *Npm1*$^{+/-}$ mice were infected with viruses as indicated (vector control, NI, NIF, NID, and NIDF) and transplanted into irradiated mice. BM cells were analyzed 8 weeks after transplantation.

H) Expression of *HoxA9* and *Meis1* in NI, NID, NIF, and NIDF cells. RNA was isolated from $EGFP^+$ BM cells prepared from the mice shown in Figure 4G. In the case of vector control mice, whole BM cells were used. Real-time PCR was performed to measure the expression levels of mHoxa9 and mMeis1.

I) Results of GSEA. The IDs of the gene sets in the MSigDB (Molecular Signature Database) are indicated.

J) Summary of GSEA.

**$P < 0.05$, ***$P < 0.001$.

Fig. 5. IDH2/R140Q negatively regulates the 5hmC modification and expression of differentiation-inducing factors

A) Identification of possible targets of IDH2/R140Q. The Venn diagram shows genes with low levels of 5hmC (white circle, 951 genes), upregulated genes (light gray,

95 genes), and downregulated genes (dark gray, 723 genes) in NIDF-AML cells. Among 27 downregulated genes with low levels of 5hmC, the expression of 13 genes was derepressed after deletion of IDH2/R140Q.

B) List of the 8 derepressed genes. Scores of lower 5hmC modification were obtained by Avadis analysis. Fold-change in gene expression (NIDF-AML cells vs. NBM cells and TAM-treated Nf(I)DF-AML cells vs. untreated Nf(I)DF-AML cells) were obtained by microarray analysis. The differentiation-inducing factors *Ebf1* and *Spib* are highlighted.

C) Expression of *Spib* and *Ebf1* in NBM cells, untreated Nf(I)DF-AML cells, and TAM-treated Nf(I)DF-AML cells was analyzed by real-time PCR.

D) 5hmC modification of *Ebf1* and *Spib* loci in NBM cells, untreated Nf(I)DF-AML cells, and TAM-treated Nf(I)DF-AML cells was analyzed by real-time PCR. (n=3 per group)

E) Model for mutant IDH2-mediated AML.