Supplemental methods

Flow cytometric analysis of cells with four mutant genes

BM cells were fixed and permeabilized using IntraPrep reagent (Beckman Coulter) and stained with anti-NGFR-APC (Biolegend), anti-FLT3-PE (eBiosciences), and anti-DNMT3A (Cell Signaling Technology) antibodies. Cells were washed, incubated with Alexa Fluor 647 anti-rabbit IgG (Molecular Probes) to detect the anti-DNMT3A antibody, and analyzed by flow cytometry.

Supplementary Figure legends

Fig. S1. Expression analysis of four mutant genes in AML cells

Permeabilized cells were stained with anti-FLT3 and anti-DNMT3A antibodies and analyzed by flow cytometry. NPMc+FLT3+ cells were gated, and DNMT3A expression was further analyzed.

Fig. S2. Flow cytometric analysis of lineage and LSC markers in NIDF-AML cells

1. Most NIDF-AML cells were positive for myeloid lineage markers. The populations of B-cells, T-cells, and erythroid lineage cells were extremely small. After deletion of IDH2/R140Q by tamoxifen treatment, the differentiation status of cells was maintained.
2. Oil- and tamoxifen-treated Nf(I)DF-AML cells were stained for several LSC markers (MCSFR, L-GMP (Lin-, Sca1-, cKit+, CD16/32+, CD34+), cKit+Gr1-, and CD34+) and analyzed by flow cytometry.

Fig. S3. (For review only)

ROS levels are upregulated in NIDF-AML cells.

Hemolyzed BM cells (2 × 106)were suspended in 1 ml of StemPro medium lacking cytokines and incubated with 2 µl of CellRox Deep Red reagent (Molecular Probes) at 37°C in 1% O2 for 30 minutes. Thereafter, cells were washed and analyzed by flow cytometry. 3 mice were analyzed for each condition.