Supporting Information

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SI Text

Methods

Gel Filtration Assay. Gel filtration assay was performed as described (1, 2). Briefly, cell lysates harvested from 293 cells transiently transfected with AE constructs were subjected to gel filtration analysis using a $\ddot{A}KTA_{FPLC}$ system with Superdex 200 gel filtration column (1.6 × 60 cm; Amersham Pharmacia) preequilibrated with running buffer [0.05 M Tris-Cl (pH 7.6), 200 mM NaCl, 3 mM EDTA, and 0.2 mM PMSF] at a flow rate of 0.5mL/min. Collected fractions were analyzed by Western blot using either M2 anti-Flag antibody (Sigma-Aldrich) or anti-myc (A-14) antibody (Santa Cruz Biotechnology).

Quantitative RT-PCR (Q-RT-PCR). The following primers have been used for Q-RT-PCR:

mCBFβ forward primer 5′ GGTTGCCTGGAGTTTGATGAG 3′

mCBFβ reverse primer 5′ GAGTTCTTCTTCGAGCCTCTTCA 3′ mCBFβ Taqman probe 5′FAM-CCCAGCAGGAAGATGCATTAGCACAAC-TAMRA 3′

Primer/probe combination against mGAPDH was obtained from Applied Biosystems.

1. Kwok C, Zeisig BB, Dong S, So CW (2006) Forced homo-oligomerization of RaRalpha leads to transformation of primary hematopoietic cells. *Cancer Cell* 9:95–108.

2. Zeisig BB, *et al.* (2007) Recruitment of RXR by homotetrameric RARalpha fusion proteins is essential for transformation. *Cancer Cell* 12:36–51.

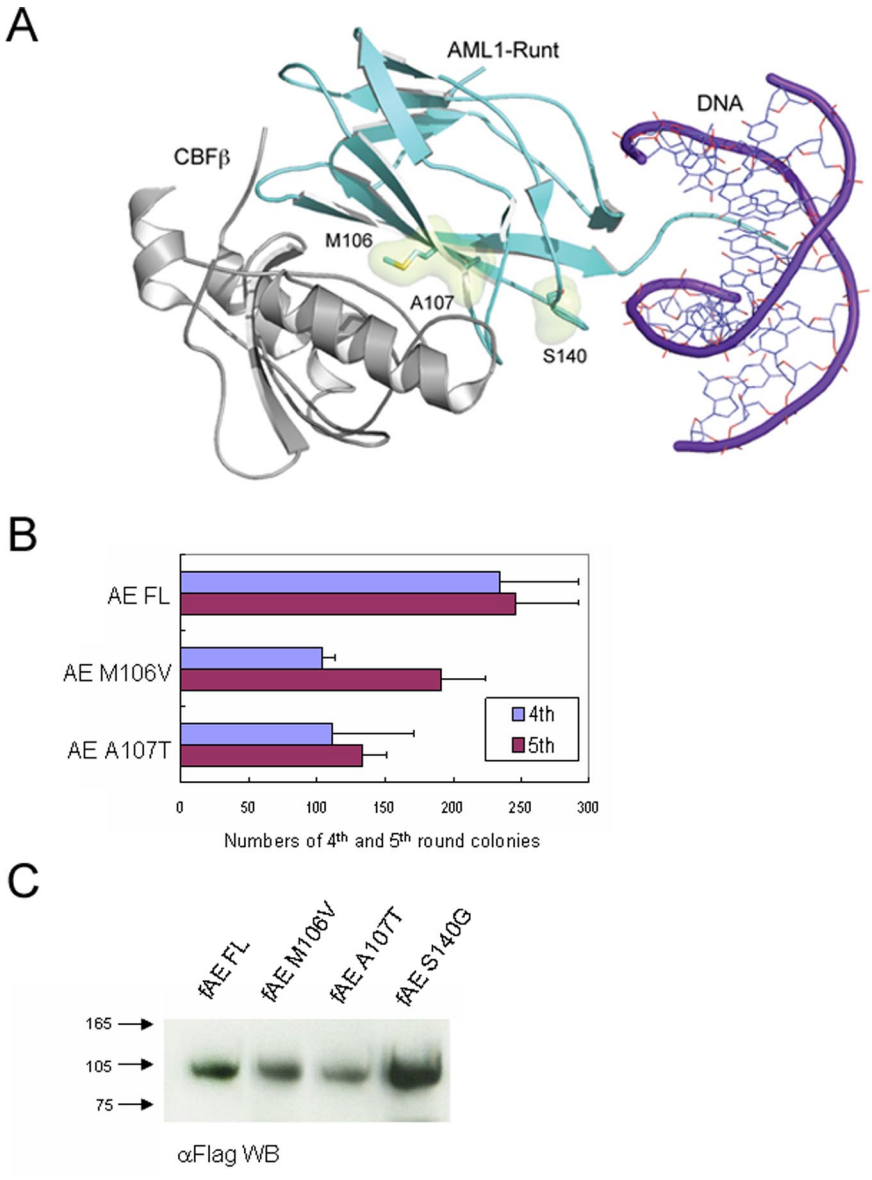

Fig. S1. Characterization of CBFβ interaction-deficient AE point mutants with enhanced self-renewal property. (*A*) Schematic representation of the complex between CBFβ (gray), the Runt domain of AML1 (cyan), and DNA (purple) based on PDB accession 1H9D. The residues M106, A107, and S140 are shown in stick representation. (*B*) Numbers of forth- and fifth-round colonies of primary bone marrow cells transduced by AE and the indicated point mutant constructs. (*C*) Western blot of 293 cell lysates transfected with indicated constructs using α-Flag antibody.

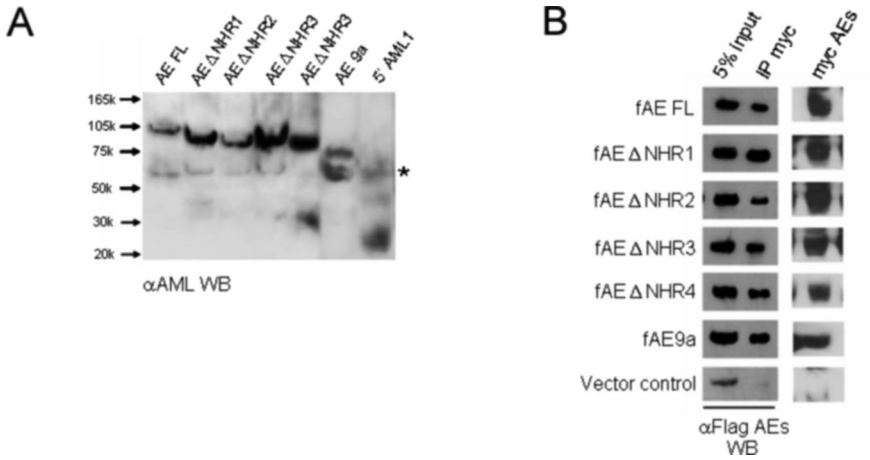

Fig. S2. Expression and dimerization properties of AE deletion mutants. (*A*) Western blot of 293 cell lysates transfected with indicated constructs using α-AML1 antibody. Asterisk indicates endogenous AML1 protein. (*B*) Coimmunoprecipitation assay between Flag-tagged and myc-tagged versions of AE constructs.

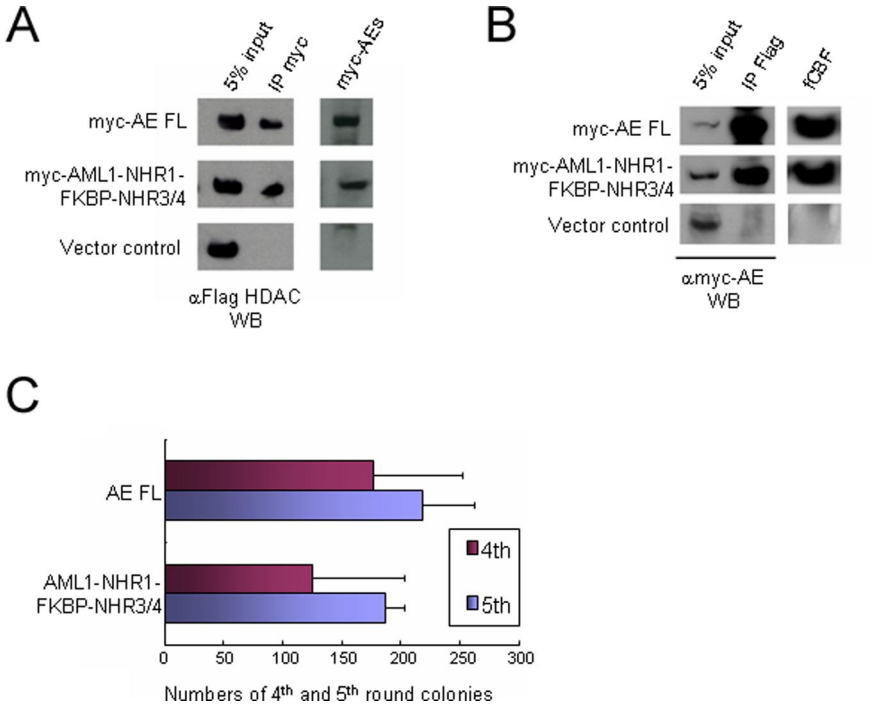

Fig. S3. Biochemical analysis of self-renewal competent AML1-NHR1-FKBP-NHR3/4 construct. Coimmunoprecipitation assay between myc-tagged AE constructs and (*A*) Flag-tagged HDAC1 or (*B*) Flag-tagged CBFβ. (*C*) Bar chart represents the corresponding numbers of forth- and fifth-round colonies transduced with the indicated constructs. Error bars indicate SD of 3 independent experiments.