SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Construction of retroviral expression vectors. Mutagenesis of Meis1a was performed in pGEM-11zf using the QuikChange site-directed mutagenesis kit (Strategene). Partial internal deletions were achieved by removing sequences flanked by two MluI sites, which had been created by mutagenesis. Progressive deletions of the carboxyl terminus were achieved by creating termination codons, using PCR.

Antibodies and immunoblot analysis. Rabbit polyclonal anti-Hoxa9 antisera was used as described (9). FlagMeis1a was detected using the M2 anti-Flag monoclonal antibody (Sigma). Antisera that bound the cytoplasmic domain of Flt3 was obtained from Upstate Biotechnology, Inc. (Lake Placid, New York), and those against c-Kit, phospho-c-Kit and phospho-MAP kinase from Cell Signaling (Beverly, MA).

Northern and Southern blotting. Northern and Southern blotting procedures were performed as described (9). EcoRI was used to digest DNA from progenitors immortalized by Hoxa9 plus Meis1 because it cuts in a single location within the provirus.

Immunoprecipitation. Radiolabeling of proteins was performed by coupled transcription/translation in vitro, using cDNA's subcloned in pGEM4zf, SP6 polymerase, [35S]-methionine, and reticulocyte lysates (Promega). For coimmunoprecipitation analysis, constructs encoding Pbx and Flag-Meis1 proteins were transcribed and

translated simultaneously. Transcription/translation reactions (10ul) were diluted with 1mL of binding buffer (10 mM Tris-HCl pH 7.5, 75 mM NaCl, 1 mM EDTA, 1 mM DTT, 1% BSA and 1% Nonidet P-40) in the presence of 3 μg M2 anti-Flag antibody. After incubation for 1hr at 4°C, 10 μl of protein A sepharose beads (Sigma) were used to bind and wash the protein-antibody complexes. Beads were washed 5 times in 1mL binding buffer. Precipitated proteins were separated by electrophoresis in SDS gels, and visualized by autoradiography.