

Figure S1. *Men1*<sup>-/-</sup> cells were infected with control retroviruses or retroviruses expressing menin. The expression of menin in the infected cells was confirmed by immunohistochemistry using menin antibody.



Figure S2. 293T cells were transfected with vectors expressing truncated menin mutants and Flag-SUV39H1 indicated. Whole-cell extracts were immunoprecipitated with anti-Flag antibody. Immunoprecipitates were analyzed by Western blot assay with antibodies against Myc and Flag epitopes. The asterisks (\*) denote nonspecific cross-reactive bands. To increase the resolution of  $\Delta$ N145 from the background, data obtained by different gel running is shown.



Figure S3. Menin associates with HDAC1 and SUV39H1.

293T cells were transfected with Myc/His-menin plus Flag-HDAC1 and Myc-SUV39H1 expression vectors as indicated. Whole-cell extracts were immunoprecipitated with anti-Flag antibody, and the immunoprecipitates were analyzed by western blotting using anti-Flag or anti-Myc antibodies. The asterisks(\*) denote IgG heavy chain bands.



menin 388-445 -----ERPGEQSQGTQSQGSALQDPECFAHLLRFYDGICKWEEGSPTPVLHVGWATFLVQSLG parafibromin 129-191 DEVLAEAKKPRIEDEECVRLDKERLAARLEGHKEGIVQTEQIRSLSEAMSVEKIAAIKAKIMA--

Figure S4. The SUV39H1 binding domains of parafibromin and menin have a remote homology. Their structures were predicted with fold recognition by threading method using the structure of paired box protein Pax5 (PDB entry: 1K78) as a template.



Figure S5. (A-C) The specificity of siRNAs targeting *Men1* or *SUV39H1* was confirmed by quantitative real-time PCR. *Men1+/+* or *Men1-/-* cells were treated with siRNAs as indicated for 24 hr and total RNA was isolated to detect the expression level of *Men1* or *SUV39H1*. *SUV39H1* siRNA did not interfere with the level of menin and vice versa. Each mRNA level was normalized to the GAPDH expression level and presented as a relative value. Error bars represent standard deviation (n = 3).



Figure S6. The cDNA microarray analysis to reveal common target genes of menin and SUV39H1. Venn diagram summarizing differentially expressed genes (up regulated genes) in *Men1*-/- MEFs vs. *Men1*+/+ MEFs and in SUV39H1 knockdown vs. control cells (top panel). Genes with fold change >1.2 were shown. Bar chart shows the gene ontology classification of the common target genes. Some genes are classified in more than two different categories.



Figure S7. Occupancy of MLL around the *Gbx2* and HoxA9 regions. Recruitment of MLL protein around the *GBX2* or *Hoxa9* was analyzed by ChIP assay. Chromatin solution was prepared from *Men1*<sup>+/+</sup> and *Men1*<sup>-/-</sup> cells and subjected to ChIP using control rabbit IgG and the antibodies against MLL ( $\alpha$ -MLL Ab) or menin ( $\alpha$ -menin Ab). Immuno-precipitated DNA was analyzed in duplicates by quantitative real time PCR and the relative enrichment of proteins was shown. The data represent the percentage of ChIP (IP/Input) and the error bars indicate the SD.