Supplementary Figure S2

(A) Biochemical fractionation of 293 cells. Cells were lysed and fractionated into soluble cytoplasmic proteins (S2) and nuclei. Nuclei were divided into two aliquots and treated with (+) or without (-) micrococcal nuclease. Nuclei were lysed and fractionated into soluble nuclear proteins (S3) and nuclear pellet (P3). hLin-9 was immunoprecipitated and detected by immunoblotting. A significant fraction of hLin-9 was released from the nuclear pellet by treatment with micrococcal nuclease demonstrating that hLin-9 is associated with chromatin.

(B) Endogenous and ectopically overexpressed hLin-9 can be crosslinked to chromatin. 293 cells or 293 cells transiently expressing flag-hLin-9 were treated with 1.1 % formaldehyde and lysed with 0.25 % Triton X-100. Nuclei were collected and soluble chromatin was prepared by sonication. hLin-9 was immunoprecipitated with anti-hLin-9 or anti-flag antibodies and detected by immunoblotting. Triton X-100 extracts of untreated cells were prepared in parallel (- crosslink). While hLin-9 was quantitatively extracted with 0.5 % Triton-X 100 from untreated cells, it was exclusively found in the chromatin fraction after crosslinking of proteins to DNA with formaldehyde.