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Supplementary MATERIALS AND METHODS

Northern Blots

Total RNA was isolated from cell lines or from primary tissues of an adult male mouse with TRIZOL (Invitrogen). Northern blots were performed with 10 μg of RNA and [□ -^{32} P] dCTP-labeled mouse or human lin-9 cDNA probes.

Cell lines

U2-OS cell clones stably overexpressing hLin-9 were generated by transfection with pLin9-IRES-neo, selection for 10 days with 800 μg/ml neomycin and isolation of individual clones. Pools stably expressing the empty pIRES-neo vector served as controls.

Chromatin Isolation

Chromatin was isolated as described previously with small modifications (Mendez and Stillman, 2000). Briefly, cells were resuspended in buffer A (10 mM HEPES, [pH 7.9], 10 mM KCl, 1.5 mM MgCl 2, 0.34 M sucrose, 10% glycerol, 1 mM DTT, 5 μg/ml aprotinin, 5 μg/ml leupeptin, 0.1 mM phenylmethylsulfonyl fluoride). NP-40 (0.1%) was added, and the cells were incubated for 5 min on ice. Nuclei (P1) were collected by low-speed centrifugation (5 min, 1,300g, 4°C). The supernatant (S1) was further clarified by high-speed centrifugation (15 min, 20,000g, 4°C). Nuclei were washed once in buffer A, and then lysed in buffer B (3 mM EDTA, 0.2 mM EGTA, 1 mM DTT, protease inhibitors as described above). Insoluble chromatin was collected by centrifugation (5 min, 1,700g, 4°C) and washed once in buffer B. The

final chromatin pellet (P3) was resuspended in sonication buffer (50 mM Tris, [pH 8.1], 10 mM EDTA, 1%SDS) and sonicated 2 x 10s in a Branson sonicator using a microtip at 10% amplitude. Chromatin was diluted 1:10 with dilution buffer (0.01 % SDS, 1.1 % Triton, 1.2 mM EDTA, 16.7 mM Tris-Cl pH 8.2, 167 mM NaCl, 10µg/ml PMSF, 10µg/ml leupeptin, 10µg/ml aprotinin) and hLin-9 was immunprecipitated with anti-hLin-9 antiserum. To release chromatin-bound proteins by nuclease treatment, cell nuclei (P1) were resuspended in prewarmed buffer A plus 1 mM $CaCl_2$ and 0.3 U of micrococcal nuclease (Sigma). After incubation at 37°C for 1 min, the nuclease reaction was stopped by the addition of 1 mM EGTA. Nuclei were collected by low-speed centrifugation and lysed according to the chromatin isolation protocol described above