SUPPLEMENTARY INFORMATION

Experimental Procedures

Nuclear extract preparation—Cells were washed with cold PBS, and then incubated at -20 °C for 5 minutes. Subsequently, buffer A containing 10 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 10 mM KCl, 0.5 mM dithiothretol (DTT), and 1 mM PMSF was added. The cytoplasmic and nuclear fractions were separated by centrifugation at 17,000 × g for 30 minutes at 4 °C. The nuclear pellets were then resuspended in buffer C containing 20 mM HEPES (pH 7.9), 1.5 mM $MgCl_2$, 420 mM KCl, 0.2 mM EDTA, 25 % glycerol, 0.5 mM DTT and 1 mM PMSF, and mixed by rotating for 1-2 hours at 4 °C. Particulate material was removed by centrifugation at 18,000 × g for 30 minutes at 4 °C. The supernatant was dialyzed against 50-100 volumes of binding buffer (12% glycerol, 60 mM KCl, 12 mM HEPES, pH 7.8, 0.12 mM EDTA, 5 mM $MgCl_2$, 5 mM DTT, and 0.1 % Triton X-100) at 4 °C for 5 hours. The nuclear extract was stored at -80 °C until use.

Protein purification by affinity chromatography—The oligonucleotide probes were synthesized and labeled with biotin at the 5' end of the forward oligonucleotide. The complementary reverse sequence was synthesized and annealed with the biotin-labeled oligomer in 10 mM HEPES (pH 7.8), 10 mM $MgCl_2$, and 0.1 mM EDTA by heating at 65 ^oC for 5 minutes followed by cooling to room temperature. Double-stranded DNA formation was verified by 3 % agarose gel electrophoresis. Five μg biotin labeled double-stranded oligonucleotides (biotin probes) were mixed with 5 mg F9 nuclear extracts in the presence of non-specific DNA competitor (non-biotin probes) to a final concentration of 250 μg/ml (the volume depended upon the concentration of nuclear protein, generally ranging from 1.5 ml to 3 ml). Binding reactions were conducted for 2 hours at 20 ^oC or overnight at 4 ^oC with continuous gentle rotation in the presence of protease inhibitor PMSF at a concentration of 1 mM. The reaction mixture was centrifuged at 2000 × g at 4 ^oC for 5 minutes to remove debris. Simultaneously, the streptavidin sepharose high performance beads (Amersham Biosciences, cat # 17-5113-01) were washed 5 times with binding buffer. The mixture of DNA-protein complexes was added to 60 μl of 100 % beads. The binding reaction was conducted for 3 hours at 4 ^oC with continuous gentle rotation. At the end of the reaction, the mixture was centrifuged at 500 × g at 4 ^oC for 5 minutes. After the beads were washed 3 times with 50 volumes of 1 × binding buffer containing 60 mM KCl and twice with binding buffer containing 150 mM KCl, 75 μl of 1 × SDS loading buffer was added. The sample was boiled for 10-15 minutes to elute sequence-specific binding proteins or interacting proteins. Following centrifugation at 15,000 × g for 10 minutes, the purified proteins were subjected to 10 % SDS-PAGE and were visualized using Coomassie blue staining. The bands present in the biotin-*FGF4* probe lane, but absent when 50×non-biotin-*FGF4* probe was included, were removed for mass spectrometric analysis as described previously (1).

RT-PCR—Specific genes were amplified with Taq DNA polymerase (Hua Nuo, Shanghai, China) in a thermocycler under a variety of conditions. PCR products were resolved on a 2 % agarose gel and visualized using ethidium bromide staining.

Supplemental Figure Legends

Supplemental Figure 1. PARP inhibitor (3AB) inhibits *FGF4* expression. (A) ES cells were cultured under the differentiation condition and treated with PARP inhibitor (3AB) for 48 hours. The *FGF4* expression level was determined by RT-PCR. (B) F9 and P19 EC cells were treated with 3AB at different concentrations for 48 hours and RT-PCR assays were conducted. The representative results of three independent experiments are shown.

Supplemental Figure 2. QPCR analysis of gene expression in F9 EC cells treated with PJ34 at different concentrations for 48 hours. A t-test was used for statistical analysis, n=3.

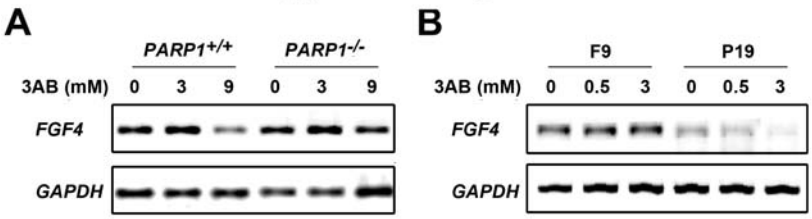
Table S1. Probes and primers used in this study.

Oligonucleotides probes		
FGF4	Forward	5'-GAAAACTCTTTGTTTGGATGCTAATGGGATACTTAAA-3'
	Reverse	5'-TTAAGTATCCCATTAGCATCCAAACAAAGAGTTTTC-3'
NC	Forward	5'-CCAAGTACCTGGAACTCCGGTAGTACCTGGAACTCCGGATGC-3'
	Reverse	5'-GCATCCGGAGTTCCAGGTACTACCGGAGTTCCAGGTACTTGG-3'
Primers used for ChIPs		
FGF4	Forward	5'-AGACTTCTGAGCAACCTCCCGAAT-3'
enhancer	Reverse	5'CAACTGTCTTCTCCCCAACACTCT3'
Sox2	Forward	5'-TTTTCGTTTTTAGGGTAAGGTACTGGGAAG-3'
enhancer	Reverse	5'-CCACGTGAATAATCCTATATGCATCACAAT-3'
FGF4	Forward	5'-GCAGCGAGGCGTGGTGAGCATCTT-3'
exon	Reverse	5'-CCCCTTCTTGTTCCGCCCGTTCTT-3'
Primers used for q-PCR and RT-PCR		
FGF4	Forward	5'-GCAGCGAGGCGTGGTGAGCATCTT-3'
	Reverse	5'-CCCCTTCTTGTTCCGCCCGTTCTT-3'
Sox2	Forward	5'-CGAGATAAACATGGCAATCAAATG-3'
	Reverse	5'-AACGTTTGCCTTAAACAAGACCAC-3'
PARP1	Forward	5'-AAGGCGGAGAAGACATTGGG-3'
	Reverse	5'-TTCATCTGTTCCATCCACCTCG-3′
GAPDH	Forward	5'-GTCGTGGAGTCTACTGGTGTC-3'
	Reverse	5'-GAGCCCTTCCACAATGCCAAA-3'

REFERENCES

1. Xu, H. M., Liao, B., Zhang, Q. J., Wang, B. B., Li, H., Zhong, X. M., Sheng, H. Z., Zhao, Y. X., Zhao, Y. M., and Jin, Y. (2004) *J Biol Chem* **279**, 23495-23503

Supplemental Figure 1



Supplemental Figure 2