# Supporting Information

Lin *et al.* 10.1073/pnas.0710725105

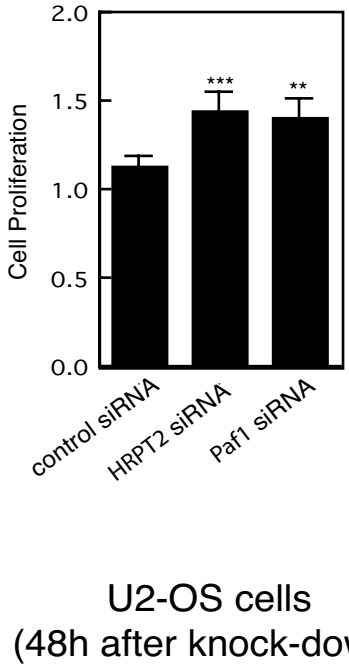


**Fig. S1.** RNA interference with parafibromin or Paf1 expression stimulates the proliferation of U2-OS cells. Human osteosarcoma U2-OS cells were treated with control-, *HRPT2*- or *Paf1*-specific siRNA as described in Methods for 48 h. Cell proliferation was estimated by a colorimetric cell proliferation assay kit (Promega). For each treatment, 5,000 cells were plated per well of a 96-well plate in sextuplicate. Presented are data representative of three individual repeats. Significance of the differences were evaluated by Student's *t* test (*** $P < 0.005$, **, $P < 0.01$).

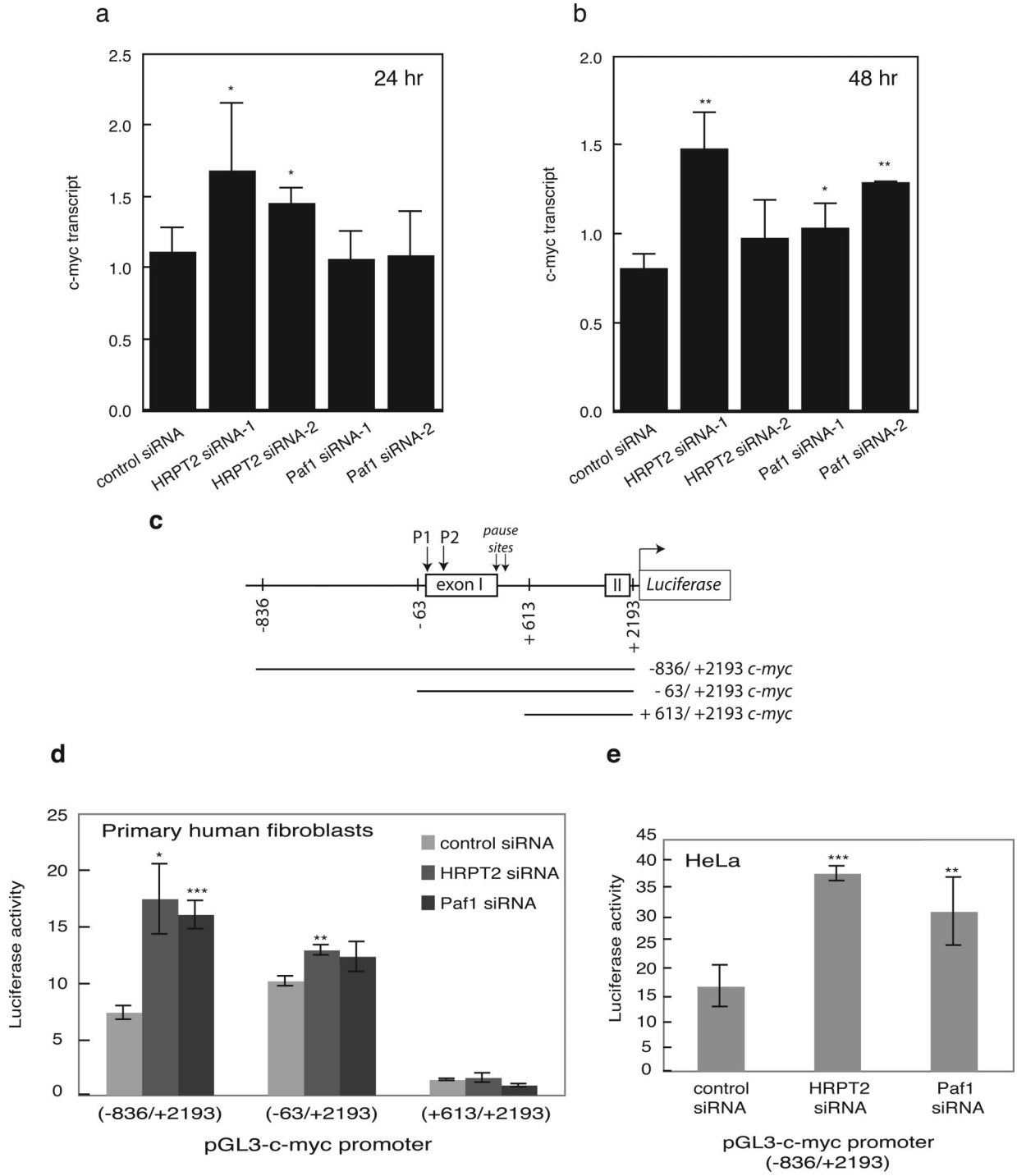


**Fig. S2.** RNA interference with parafibromin or Paf1 expression up-regulates the expression of *c-myc* transcript in HeLa cells and enhances the human *c-myc* gene promoter. (*A* and *B*) HeLa cells were treated with control siRNA, *HRPT2*- or *Paf1*-specific siRNA for 24 h (*a*) or 48 h (*b*) and the expression of human *c-myc* mRNA was examined by quantitative real-time RT-PCR analysis. Relative expression of *c-myc* transcripts was normalized to the level of $\beta$-*actin* mRNA. Data shown are averaged from a pool of three independent biological repeats, each with three technical repeats, for a total of nine technical repeats in the averaged pool. (*c*) Schematic diagram of three human *c-myc* promoter luciferase reporter constructs used ($-836/+2193$, $-63/+2193$, and $+613/+2193$) with the positions of the major P2 and minor P1 promoter start sites indicated, along with the position of the transcriptional pause sites, exon I and a portion of exon II (II), with base numbering below. Drawing not to scale. (*d*) Primary human fibroblast (NHDF) cells acutely transfected with the indicated luciferase reporter constructs in the presence of control siRNA, *HRPT2*- or *Paf1*-specific siRNA for 48 h. The average relative luciferase activities, normalized by *Renilla* luciferase activity, are represented as bars. Data shown is representative of three independent experiments with similar results. (*e*) The average relative luciferase activity in HeLa cells of the $-703/+2193$ *c-myc* promoter construct is shown after treatment with control siRNA, *HRPT2*-, or *Paf1*-specific siRNA for 48 h. The significance of the differences were evaluated by Student's *t* test (***, $P < 0.005$; **, $P < 0.01$; *, $P < 0.05$).

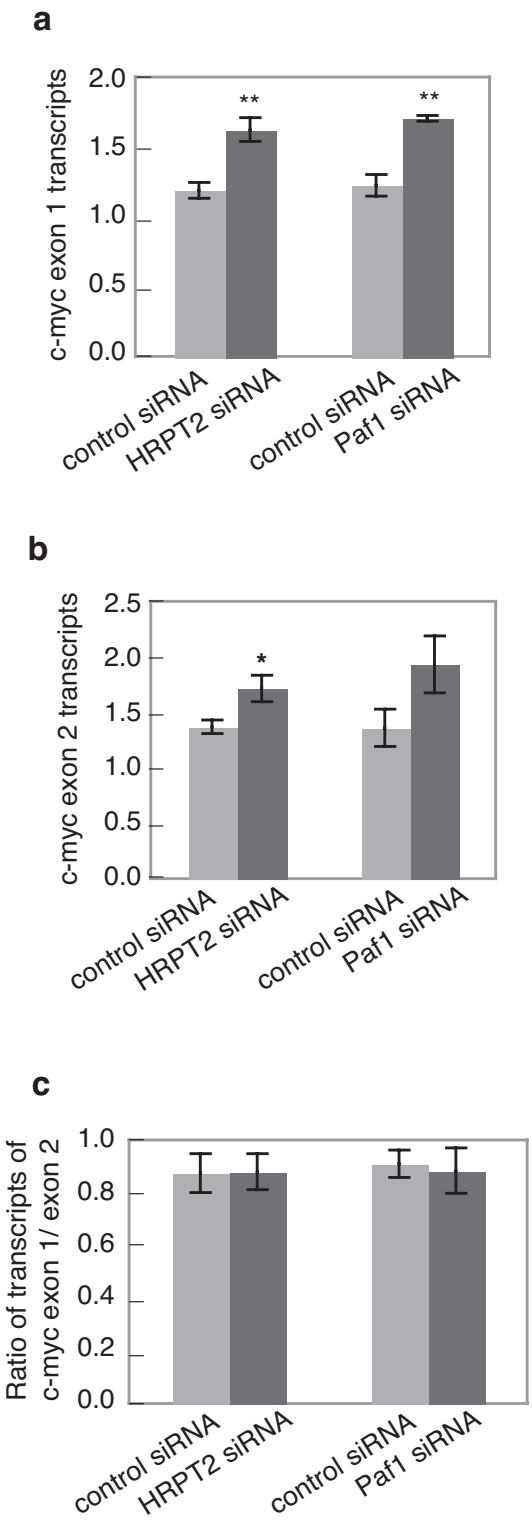


**Fig. S3.** RNA interference with parafibromin or Paf1 expression up-regulates *c-myc* exon 1- and exon 2-containing transcripts in HeLa cells to similar extents (*a* and *b*) Twenty-four hours after culture, HeLa cells were treated with control siRNA, *HRPT2*- or *Paf1*-specific siRNA for 48 h and the expression of human *c-myc* exon 1- and exon 2-containing transcripts were measured by quantitative real-time RT-PCR analysis. Relative expression of human *c-myc* exon 1- and exon 2-containing transcripts were normalized by *β-actin* transcript levels. (*c*) Ratio of relative expression of c-myc exon 1 to exon 2 among control siRNA, *HRPT2*- or *Paf1*-specific siRNA treated groups were calculated. Data shown are representative of two independent experiments. The significance of the differences were evaluated by Student's *t* test (**, $P < 0.01$, *, $P < 0.05$).

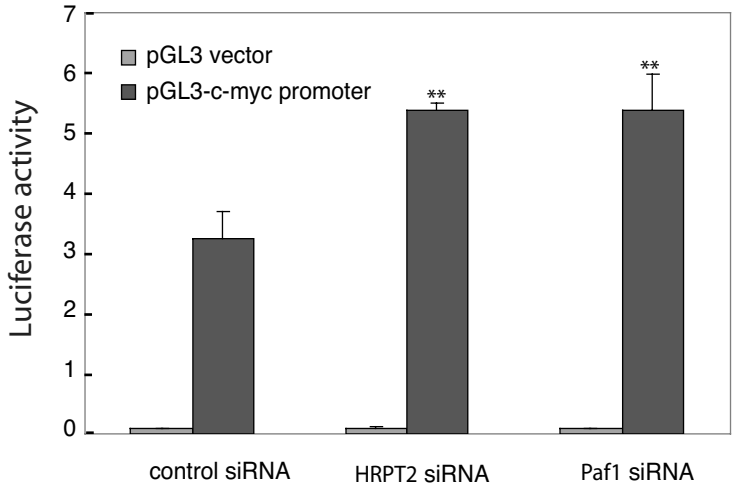


**Fig. S4.** RNA interference with parafibromin or Paf1 expression stimulates *c-myc* promoter activity in HeLa cells. The average relative luciferase activity in HeLa cells transfected with either the promoter-less pGL3 Basic control vector or the −703/+2193 human *c-myc* promoter construct (in pGL3) is shown after treatment with control, *HRPT2*- or *Paf1*-specific siRNA for 48 h. The significance of the differences were evaluated by Student's *t* test (**, $P < 0.01$).

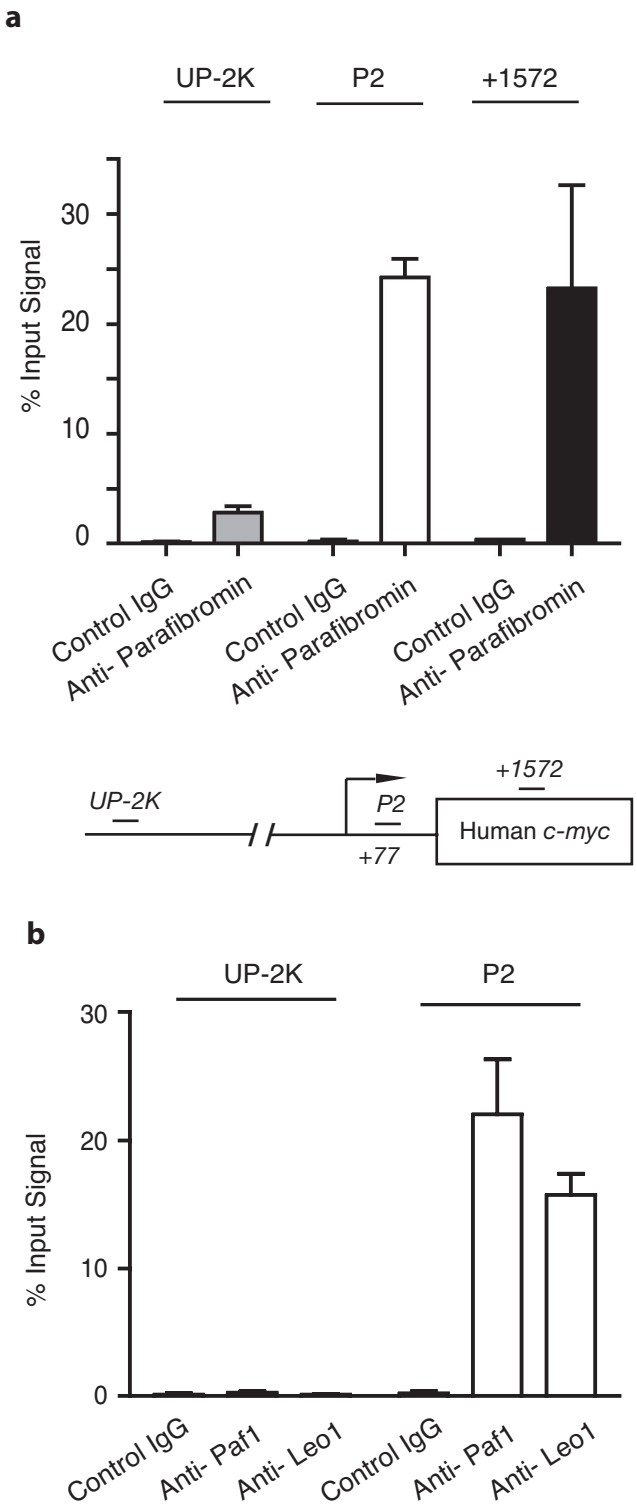


**Fig. S5.** The parafibromin tumor suppressor directly associates with the *c-myc* promoter in native HeLa cells. (*a*) Chromatin immunoprecipitation was performed in HeLa cells using control IgG and anti-parafibromin antibodies, with results analyzed by quantitative real-time PCR using primers as described in Methods, and with results expressed as the percentage of input signal. Below is shown a diagram of the human *c-myc* promoter, with the transcriptional start site (P2) denoted by an arrow, and the PCR fragments amplified for the ChIP assay shown as horizontal bars at 2 kB upstream from *c-myc* (UP-2K), P2 (+77) and + 1572. Note figure not drawn to scale. (*b*) Chromatin immunoprecipitation from HeLa cells using control IgG, anti-Paf1, or anti-Leo1 antibodies as indicated (with results analyzed by quantitative real-time PCR using primers as shown), with results expressed as the percentage of input signal. Standard error bars indicate the scatter of data pooled from three biological repeats, each with two technical repeats, for each condition.

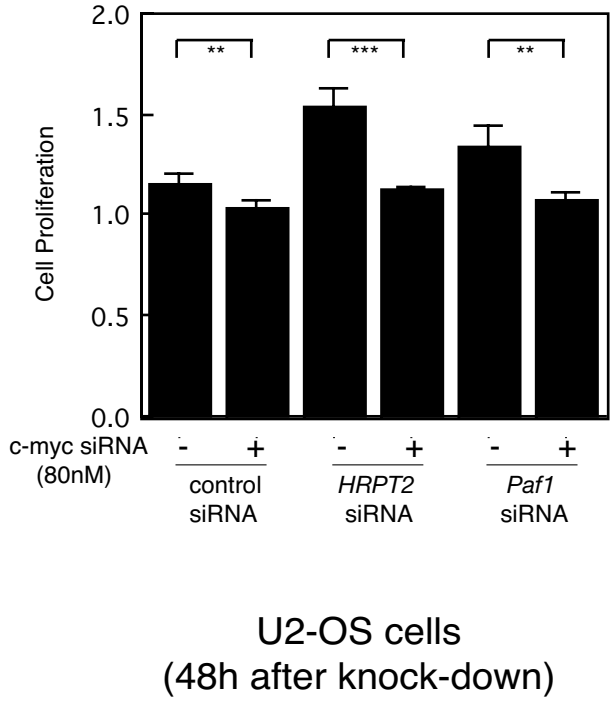


**Fig. S6.** RNA interference with c-myc expression blocks the proliferative response to parafibromin or Paf1 knockdown in U2-OS cells. Human osteosarcoma U2-OS cells were treated with control-, *HRPT2*- or *Paf1*-specific siRNA in the absence or presence of 80 nM of *c-myc*-specific siRNA as described in Methods. Forty-eight hours after treatment, cell proliferation was estimated by a colorimetric assay as described in *Methods*. Data are representative of three individual repeats with similar results. Significance of the differences were evaluated by Student's *t* test (*** $P < 0.005$, **, $P < 0.01$).

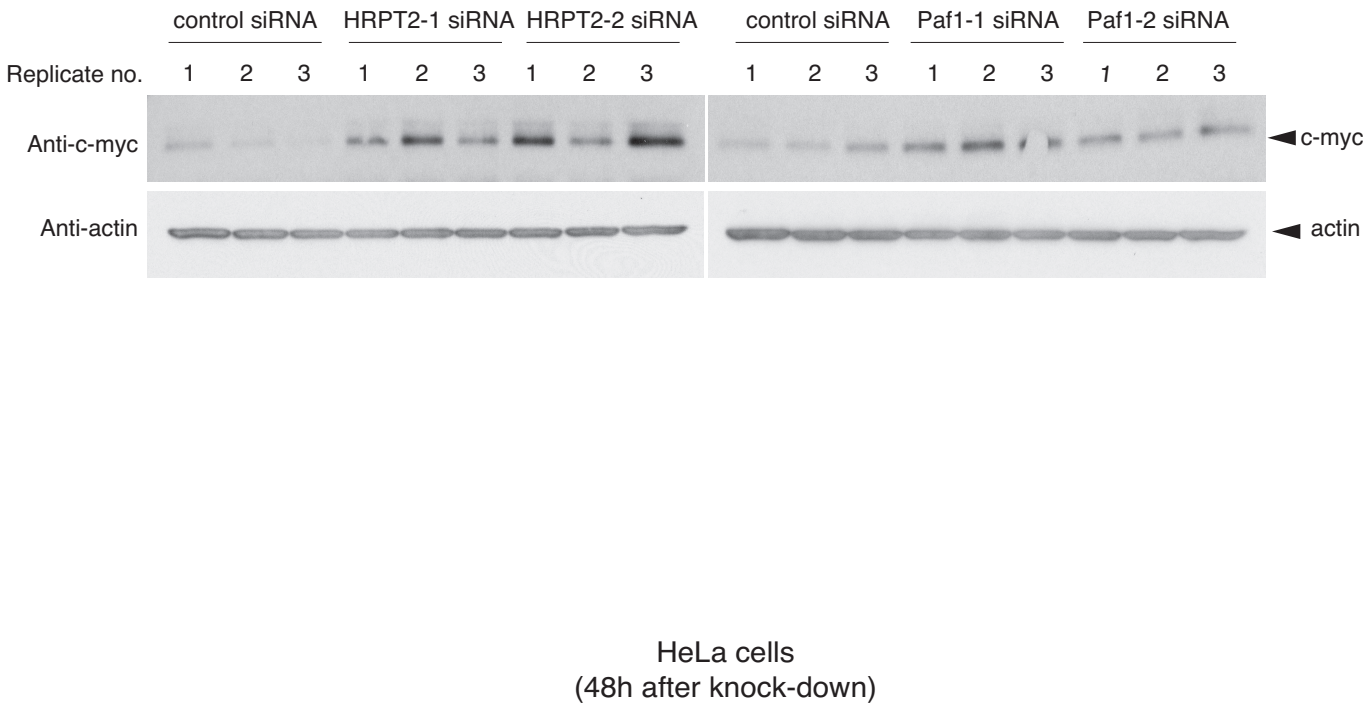


**Fig. S7.** Up-regulation of c-myc protein in following RNAi-mediated inhibition of *HRPT2* or *Paf1* expression. HeLa cells were treated with control siRNA or one of two *HRPT2*- or *Paf1*-specific siRNAs for 48 h and cells were then subjected to immunoblot analysis using anti-c-myc (*Upper Panel*) or anti-$\beta$-actin (*Lower Panel*) antibodies. After quantification of c-myc expression was carried out by quantitative ECL, and normalized to $\beta$-actin expression, the results were incorporated into the pooled data of Fig. 2 *B* and *C*. Shown are immunoblot results for three replicates (biological repeats) for each condition.