

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Additional characterization of IPTG-inducible shRNA expression in Rh30 cells. A, Western blot analysis of four 12q13-14 amplicon-negative RMS cell lines compared to 12q13-14 amplicon-positive Rh30 cells stably expressing IPTG-inducible NT control shRNA or three different shRNAs targeting CDK4. Rh30 cells were treated with 75 μ M IPTG for 48 h. B, The lowest IPTG concentration inducing maximal knockdown of CDK4 is 75 μ M. Rh30 cells stably expressing IPTG-inducible shRNA #2 targeting CDK4 were treated with vehicle or the IPTG concentrations indicated for 48 h. C, IPTG reduces CDK4 mRNA levels in Rh30 cells in a dose-dependent manner. Data represent the mean \pm SD of quadruplicate samples from three independent experiments. * $p < 0.0005$, ** $p < 0.00005$ by Student's t-test. D, Levels of MYH and myogenin are unaffected by CDK4 knockdown. Rh30 cells stably expressing IPTG-inducible shRNA #2 or #3 targeting CDK4 were treated with vehicle or 100 μ M IPTG for 5 days. Media containing IPTG was replenished on day 3.

Supplemental Figure 2. Depletion of CDK4 represses the proliferative and transformative capacity of non-amplified fusion-positive RMS cells. Rh41 cells stably expressing IPTG-inducible NT control shRNA or two different shRNAs targeting CDK4 treated with vehicle, 10 μ M IPTG, or 75 μ M IPTG demonstrate reduced CDK4 in a dose-dependent manner at the (A) protein and (B) mRNA levels by Western blot and qPCR analysis, respectively. qPCR data represent the mean \pm SD of quadruplicate samples from three independent experiments. C, Cell proliferation and (D) focus formation are abrogated upon IPTG-inducible knockdown of CDK4.

Data are the mean \pm SD of triplicate samples from two independent experiments. * $p < 0.0005$, ** $p < 0.00005$ by Student's t-test.

Supplemental Figure 3. Further evaluation of CDK4/6 inhibitor sensitivity in fusion-positive and fusion-negative RMS cells. A, PD0332991 attenuates viability of fusion-positive RMS cells. B, LEE011 fails to affect most fusion-negative RMS cell lines. C, Doxycycline-inducible overexpression of CDK4 rescues viability of Rh41 cells treated with PD0332991. Cells were treated with a range of doxycycline concentrations in the presence of vehicle or 1 μ M PD0332991. Data represent the mean \pm SD of five replicates from three independent experiments. * $p < 0.05$ by Student's t-test.