

Supplementary Figure S3

hLin-9 does not inhibit cell cycle progression

(A) Expression of hLin-9 in individual U2-OS clones stably transfected with an hLin-9-IRES-neomycin plasmid was analyzed by immunoprecipitation followed by immunoblotting. Untransfected U2-OS cells and U2-OS cells transfected with an empty IRES-neo plasmid were analyzed in parallel. (B) The DNA content of the indicated asynchronously growing U2-OS cell lines was determined by FACS. (C) The indicated U2-OS cell lines were transiently transfected with a p16 expression plasmid to activate endogenous pRB. Forty-eight hours after transfection, the DNA content was determined by FACS. Shown is the absolute change in the number of cells in G1 compared to cells transfected with an empty plasmid. These data in this Figure show that that cell cycle inhibition after activation of

endogenous pRB by p16 is comparable in control cells and in U2-OS clones expressing high levels of hLin-9.