Supplemental Figures and Legends:

Supplemental Figure 1. Cumulative viability score of siRNA knockdowns. Each siRNA knockdown from Figure 1B is color coded to allow the identification of each gene on graph.

Supplemental Figure 2. Spheroid morphology of control and gene knock down cells. Micrographs of iOvCa147-E2, HEY, and OVCAR8 spheroids knocked down for selected genes. The images depict the effects of loss of viability caused by depletion of key genes identified in our miniscreen (Dyrk1A, E2F5, $p57^{Kip2}$ and p130) in contrast to genes that have limited effects on viability (Lin9 and p107). Non-infected cells, and those infected with a control shRNA expressing lentivirus serve as controls. The scale bar equals 1 mm. Note that not all cell lines generate the same morphology of spheroids.

Supplemental Fig. 3. Reattachment of control and gene knockdown spheroid cells after 72 hours. Representative images of reattached HEY, iOvCa147E2, iOvCa129, iOvCa185 and OVCAR8 spheroids depleted of the indicated genes. The spheroids were replated onto regular tissue culture plasticware following 72 hours in suspension and allowed to attach and grow for a further 24 hours before being stained with HEMA3 and quantitated.

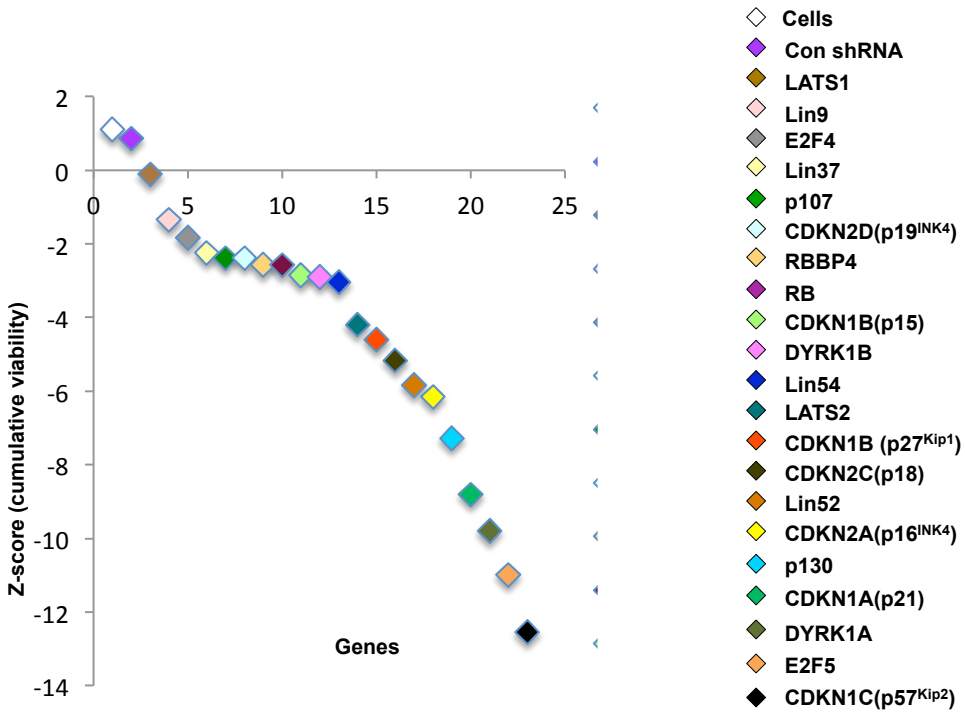
Supplemental Figure 4. Relative protein expression levels of Dyrk1A and Dyrk1B in cell lines used in this study. The relative expression of Dyrk1A and Dyrk1B in

adherent (A) or spheroid (S) culture conditions was determined by western blotting. OVCAR3 cells are known to contain a genetic amplification of the Dyrk1B encoding gene and were employed as a control for Dyrk1B expression. SmartPool siRNAs specific for Dyrk1A, Dyrk1B, or a non-targeting control were transfected into OVCAR3 cells and these extracts were run on each blot as a standard for expression and the specificity of knock downs.

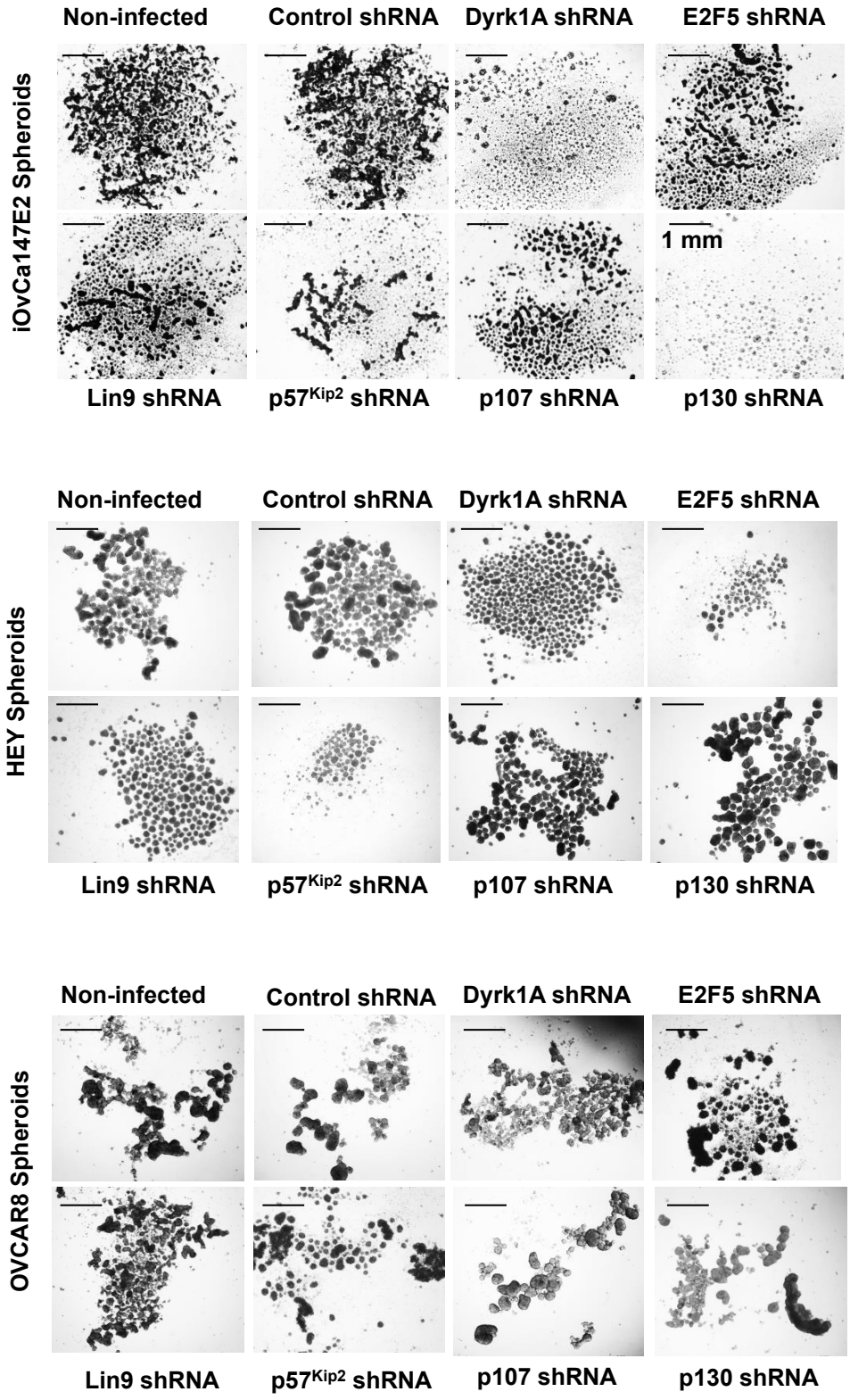
Supplemental Figure 5. Expression and function of CIP/KIP proteins in non-adherent culture. Expression levels of the CKI proteins **(A)** $p57^{Kip2}$ and **(B)** $p27^{Kip1}$ in the cell lines used in this study were determined by western blotting extracts from adherent (A) and spheroid (S) cultures. SmartPool siRNA against $p57^{Kip2}$ and a non-targeting control were transfected into HEY cells and the extracts were run on each blot as a standard for expression.

Supplemental Figure 6. Flow cytometry analysis of p130 depleted cells. Cells stably depleted of p130 by shRNA knock down, or a non-targeting control, in iOvCa147 cells were stained with propidium iodide and analyzed by flow cytometry. The proportion of cells in the relevant phases of the cell cycle are indicated along with sub-2N cellular debris and greater than 4N aneuploid cells. Note the accumulation of debris in p130 knock down specifically at 48 hours.

Supplemental Fig. 1



Supplemental Fig. 2



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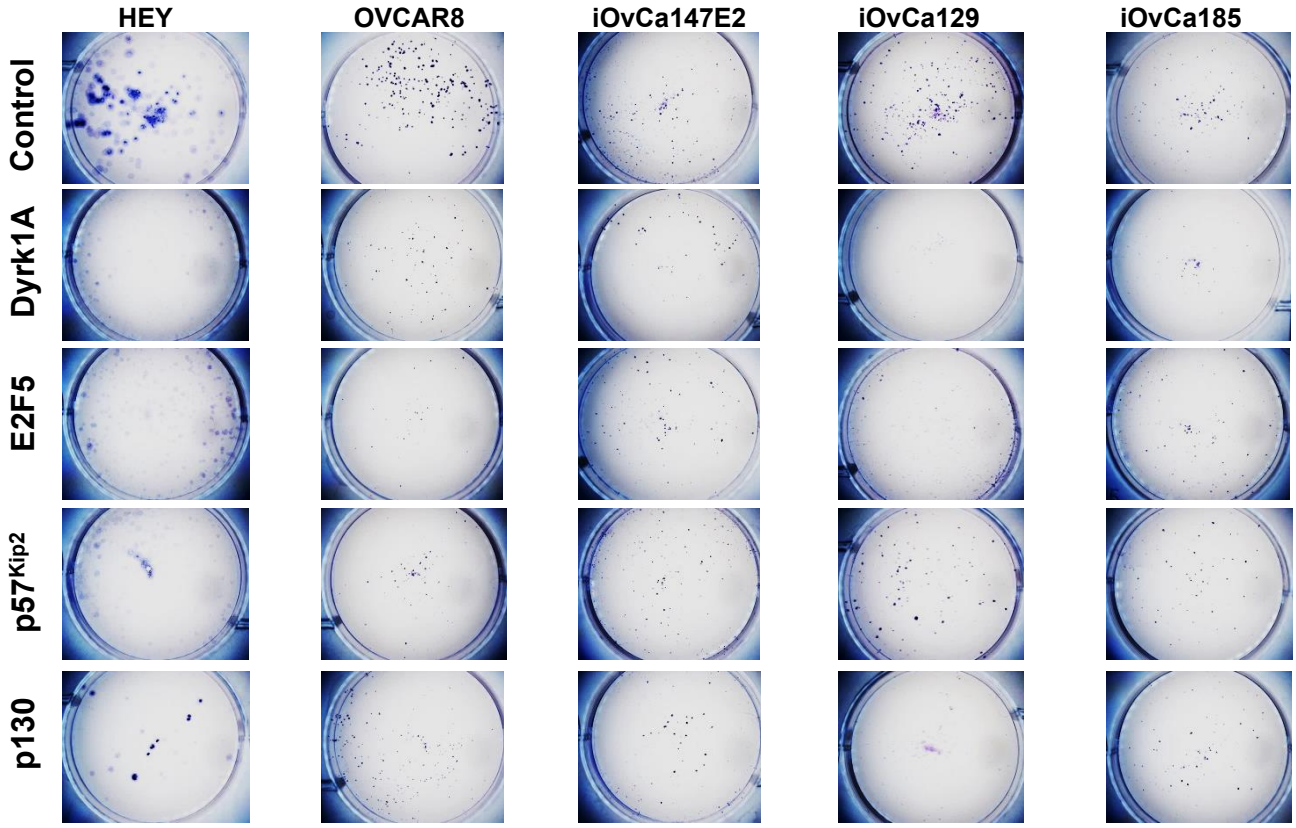
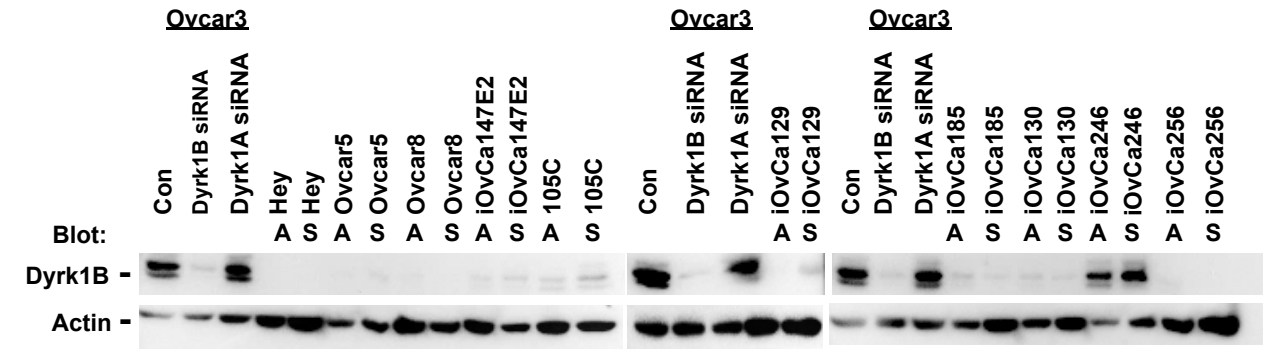


Supplemental Fig. 4

A



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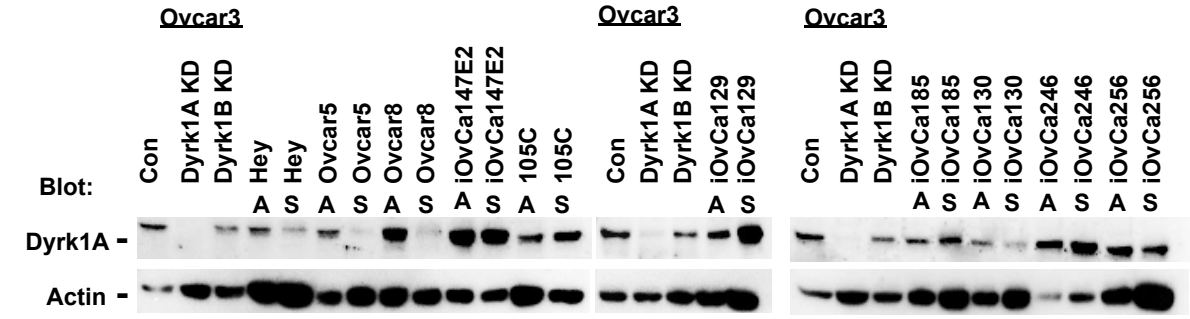
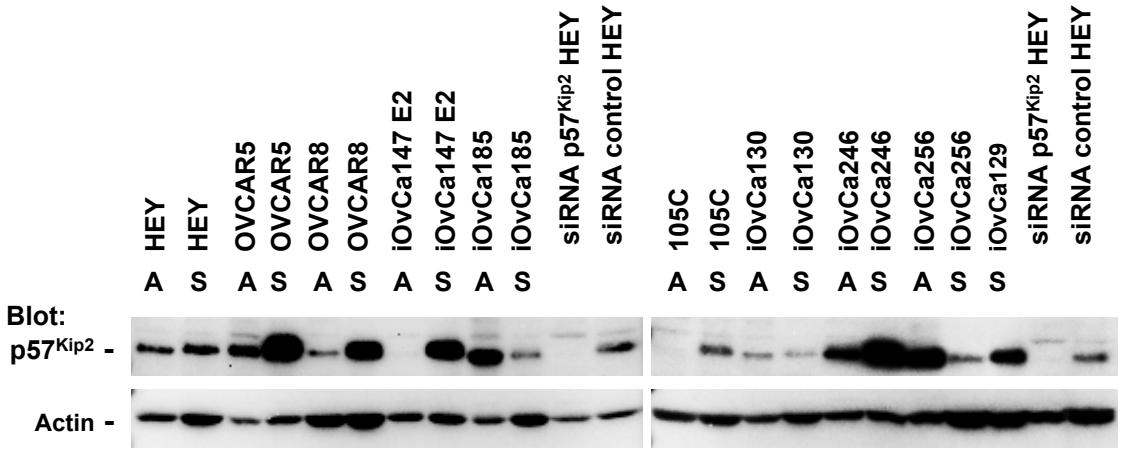


Supplemental Fig. 5

A



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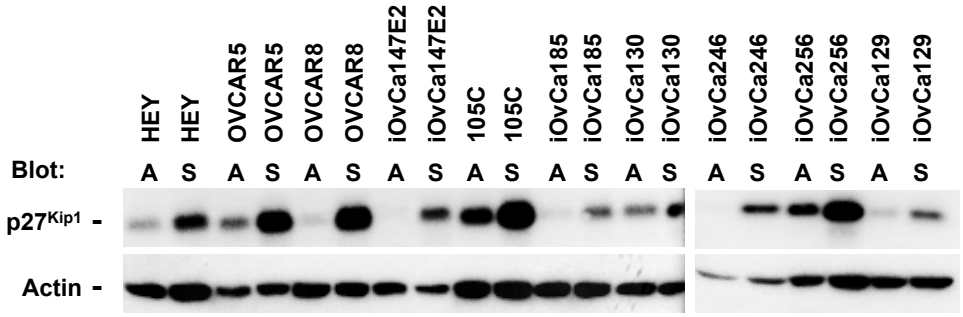

Supplemental Fig. 6