

**Figure S4.**

HMGA2 regulates S10 phosphorylation and protein levels of p27 via AKT activity. **A**, JHH-4 cells expressing Dox-responsive shRNA HMGA2#1 and the control shRNA (NT) were incubated with (+) or without (-) Dox for 4 days and examined by immunocytochemistry using a p27 antibody. The nuclei were stained with DAPI. Scale bar: 20  $\mu$ m. **B**, JHH-2 cells expressing Dox-responsive shRNA HMGA2 #1 and #2 were incubated with (+) or without (-) Dox for 4 days and examined by Western blot analysis using the indicated antibodies (Left). HLF cells expressing the shRNA HMGA2#1 were similarly examined (right). **C**, Cells were treated with LY294002 at the indicated doses for 24 h and examined as in **B**. The p27 and p-p27(S10) band intensities were quantified with ImageJ and are shown relative to the controls after normalization with the corresponding intensities of the  $\beta$ -actin loading control. The p-p27(S10) intensities were also normalized with the values of p27 and are shown in parentheses. **D**, Cells were treated with the AKT1 inhibitor, A-674563, at the indicated doses for 6 h and examined as in **C**.  $\alpha$ -tubulin: the loading control. **E**. The fractionated cell lysates from JHH-4/shRNA(NT) in Fig. 3G were analyzed by Western blotting using anti-p-p27(S10) antibody. W; whole, N; nuclei, C; cytosol.