SUPPLEMENTARY METHODS

Western Blot Analyses and Quantitative Reverse Transcription - Polymerase Chain Reaction (RT-PCR)

The blots were analyzed against pan-RAS (1:500 dilution), KLF5 (1:2,500 dilution), β-actin (1:5,000 dilution), phospho-ERK1/2 (1:1,000 dilution) or ERK1/2 (1:1,000 dilution) using the respective primary antibodies. Quantitative RT-PCR was performed as previously described[1], using primers designed for KLF5 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

Cell proliferation and anchorage-independent focus formation assays

Cells were seeded onto 6-well plates in medium supplemented with 10% FBS at a density of 5×10^4 cells per well, and the medium was replaced daily. The cells were trypsinized each day and counted using a Z1 Coulter counter (Beckman Coulter, Fullerton, CA). Anchorage-independent focus formation assays were also performed as previously reported [1]. Cells with a density of 1×10^3 per 10 cm-plate were seeded in a 0.3% top-agar suspension overlaid on a 0.5% bottom agar layer. The cells were fed with fresh medium with 10% FBS every three days and incubated at 37 ºC in 5% CO_2 atmosphere. The colonies were then counted at the end of three weeks of incubation.

Immunohistochemistry image quantification

Intensities of KLF5 staining in immunohistochemical images of tumor and normal tissues were quantified and compared using MetaMorph Image Analysis software (Molecular Devices, Downington, PA). For each image frame (see for example, Figure 7A), a constant intensity threshold was first set to distinguish between KLF5-expressing and non-expressing cells.

Staining intensities and areas of KLF5-expressing cells were then measured to obtain the ratio of staining intensity over area for each cell. Relative KLF5 intensity was then calculated by averaging the above ratios for all KLF5-expressing cells in the image frame.

	Age/Sex	Tumor Staging			K-Ras Genotype
Patient 1	**79F**	**T3**	**N2**	**Mx**	**G13D**
Patient 2	**51F**	**T3**	**N1**	**Mx**	**G12D**
Patient 3	**59M**	**T2**	**N0**	**Mx**	**WT**
Patient 4	**45F**	**T3**	**N2**	**Mx**	**G13V**
Patient 5	**70M**	**T1**	**N0**	**Mx**	**G13V**
Patient 6	**76M**	**T2**	**N0**	**Mx**	**G13V**
Patient 7	**72M**	**T3**	**N2**	**Mx**	**A- G13V**
					B- G13V

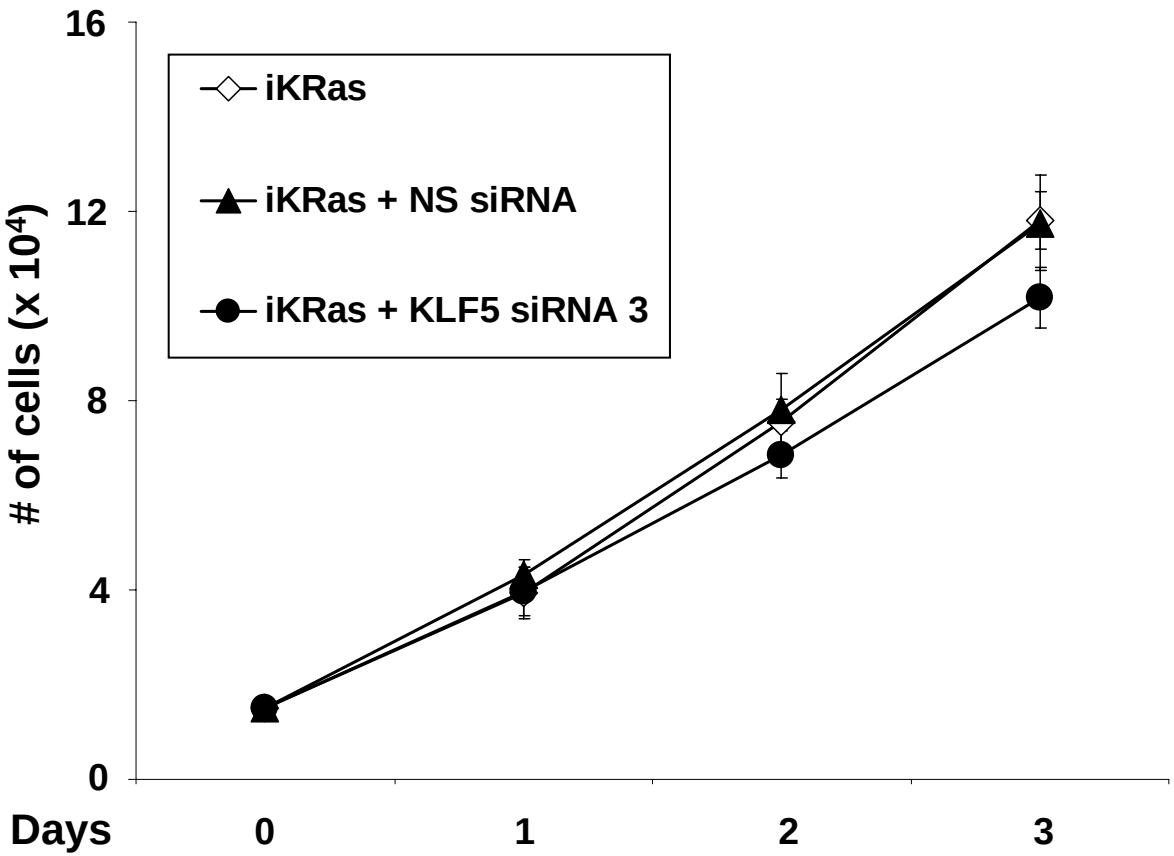
SUPPLEMENTARY TABLE

Clinical data on resected tumor samples from patients with colorectal carcinoma. Patient 7 had two primary tumors. Tumor staging data provide information regarding the extent of primary tumor (T), spread to regional lymph nodes (N) and degree of metastasis (M). Codons 12, 13 and 61 of the KRAS gene were also sequenced and noted for each tumor. Mx is without metastasis. WT indicates wild type.

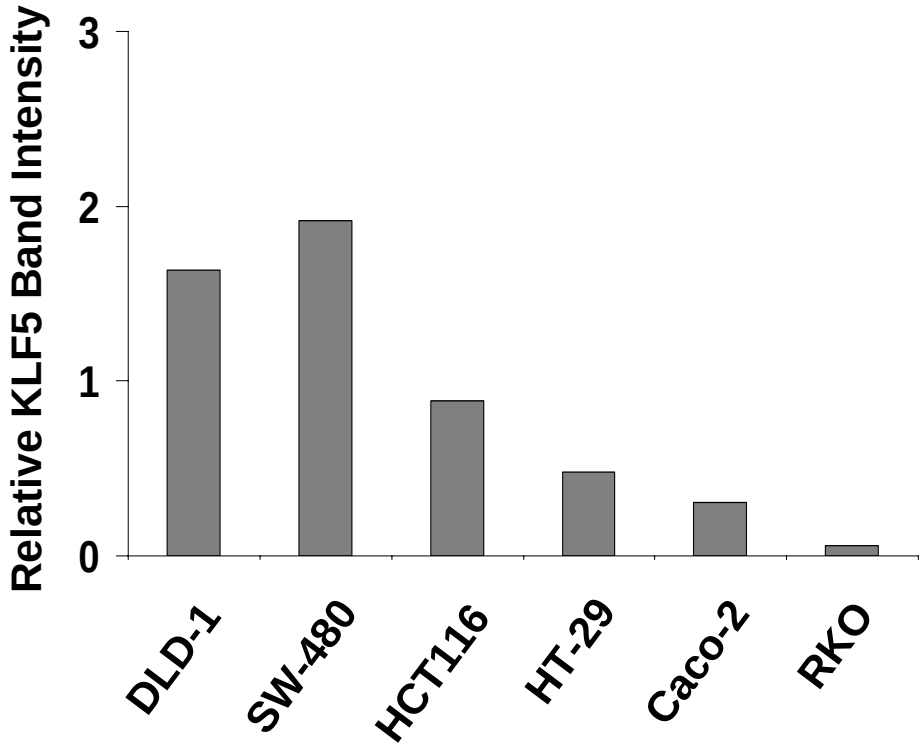
SUPPLEMENTARY REFERENCES

1. Nandan MO, Yoon HS, Zhao W, Ouko LA, Chanchevalap S, Yang VW. Krüppel-like factor 5 mediates the transforming activity of oncogenic H-Ras. Oncogene 2004;23:3404-13.

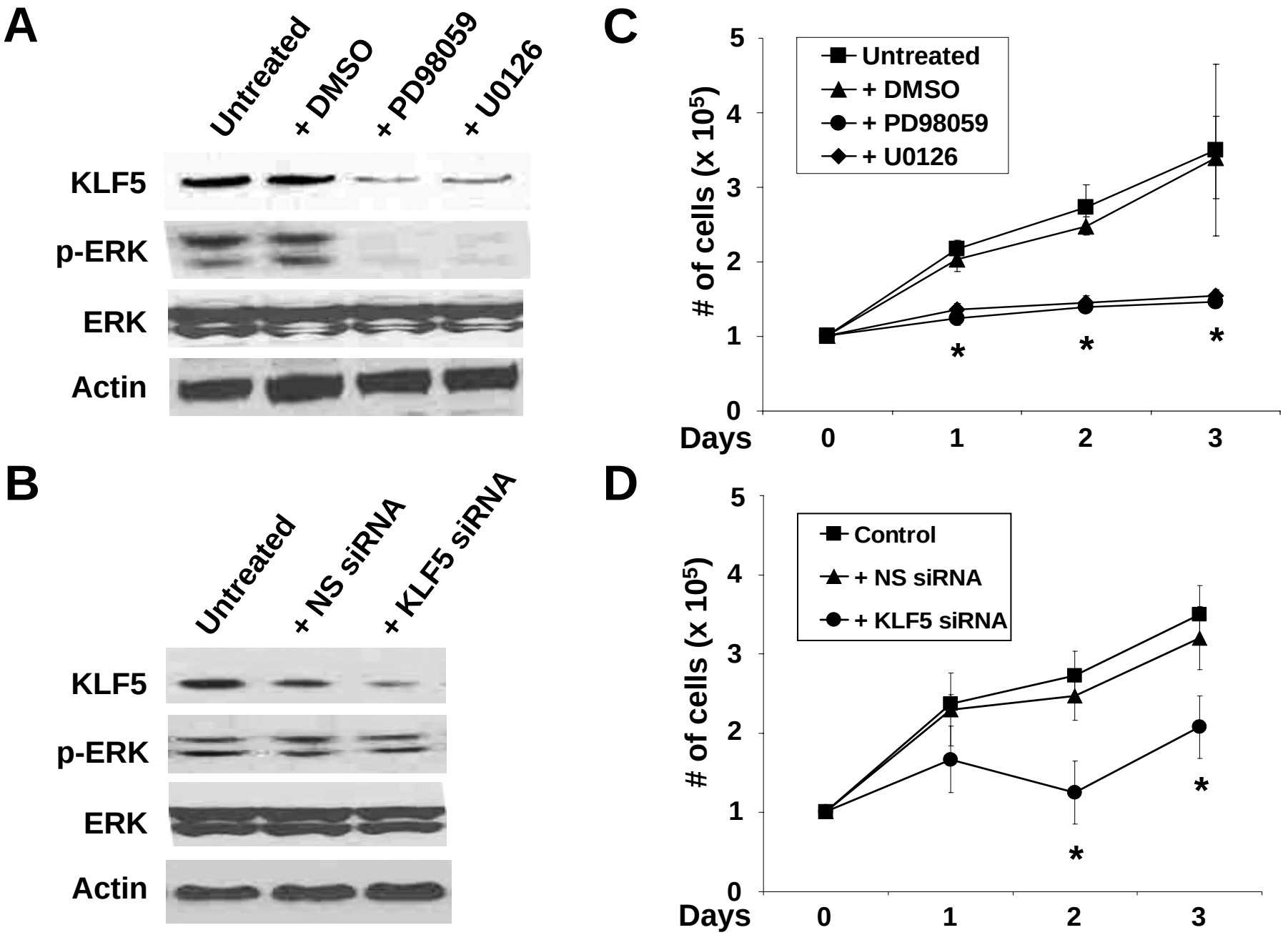
2. Du JX, Yun CC, Bialkowska A, Yang VW. Protein inhibitor of activated STAT1 interacts with and up-regulates activities of the pro-proliferative transcription factor Krüppel-like factor 5. J Biol Chem 2007;282:4782-93.



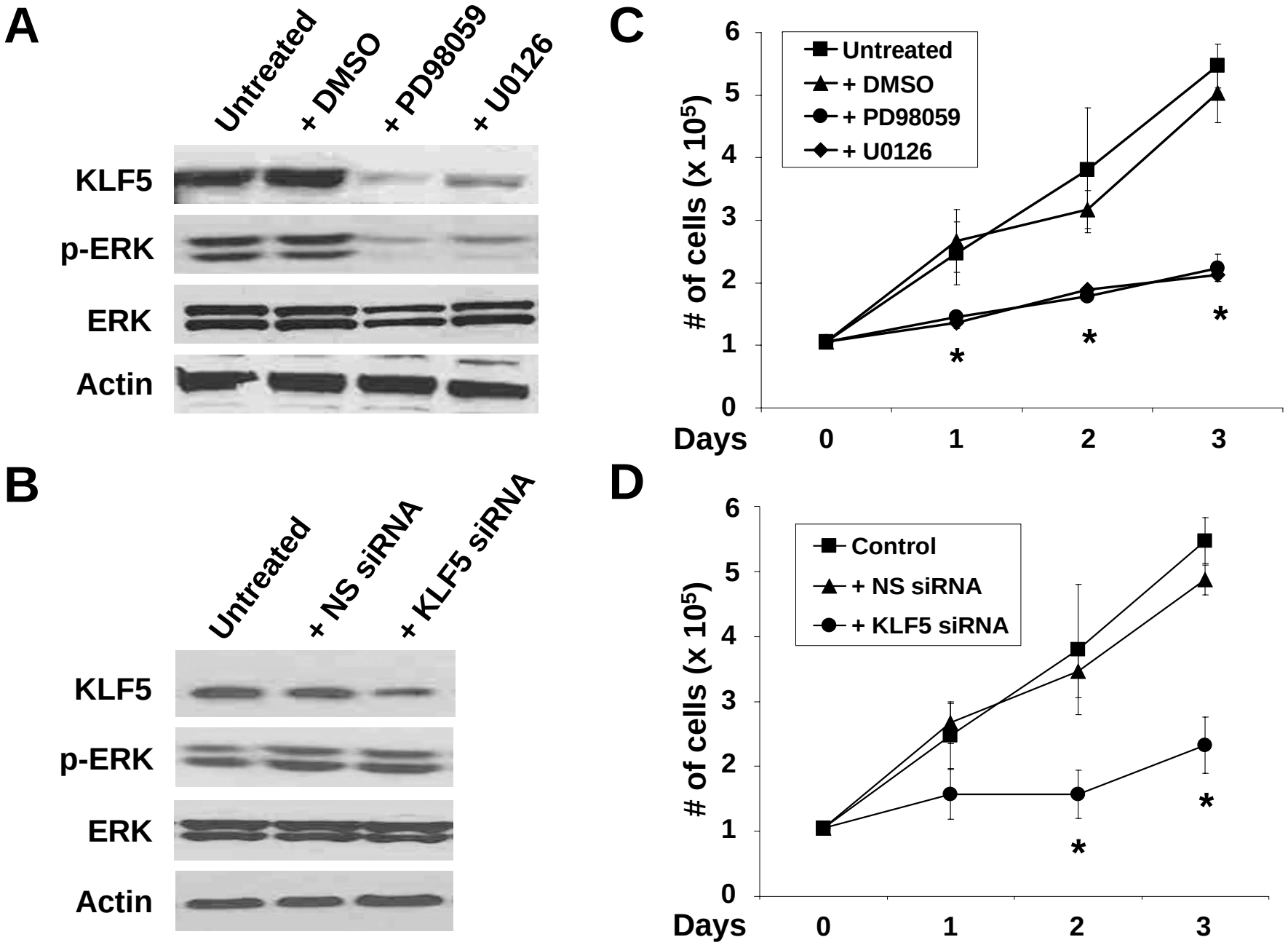
Supplementary Figure 1



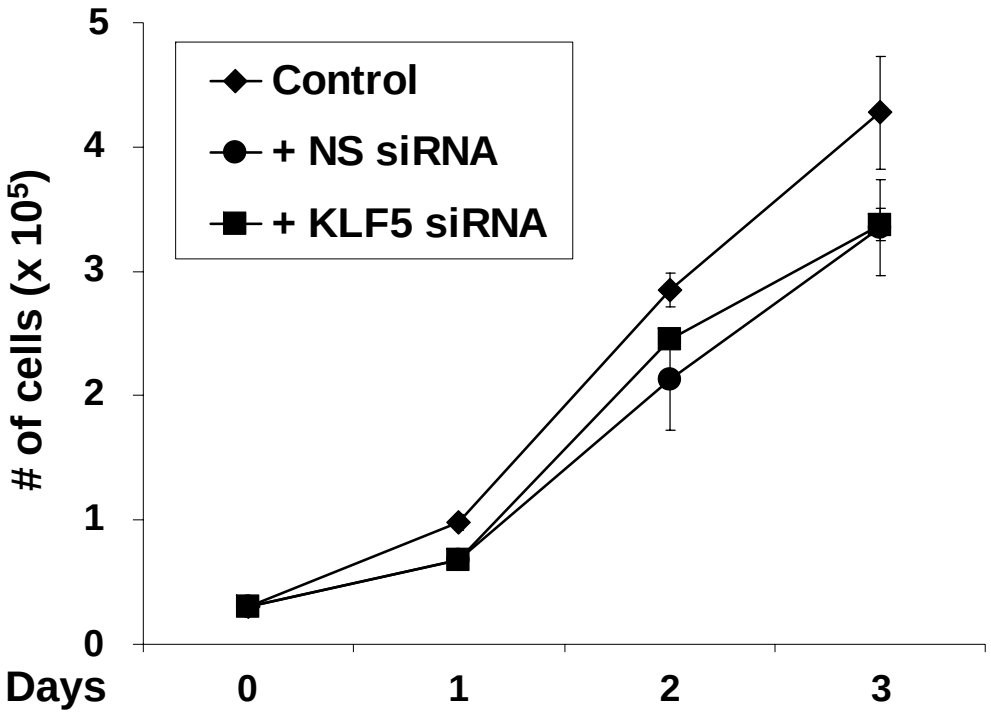
Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4



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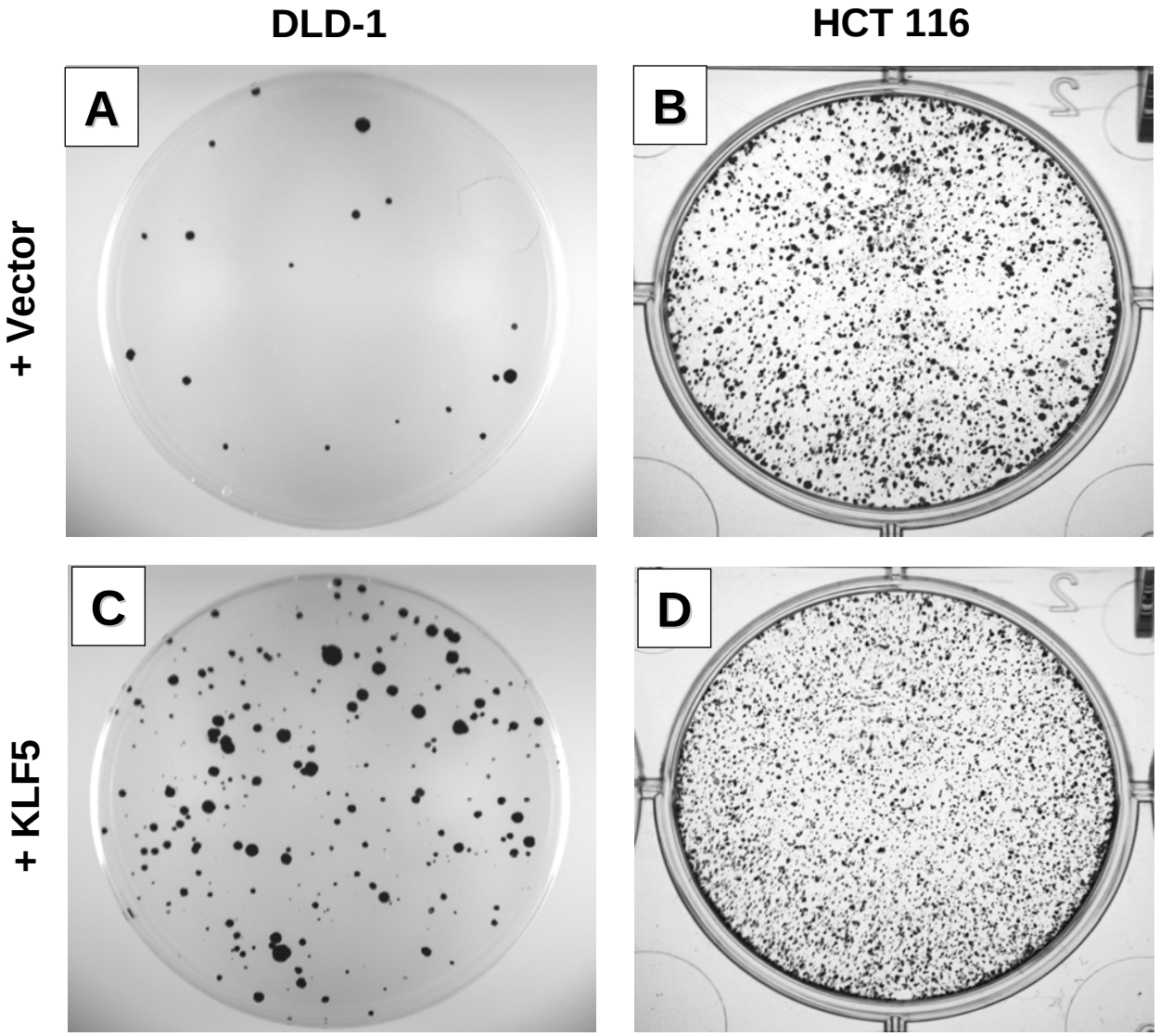

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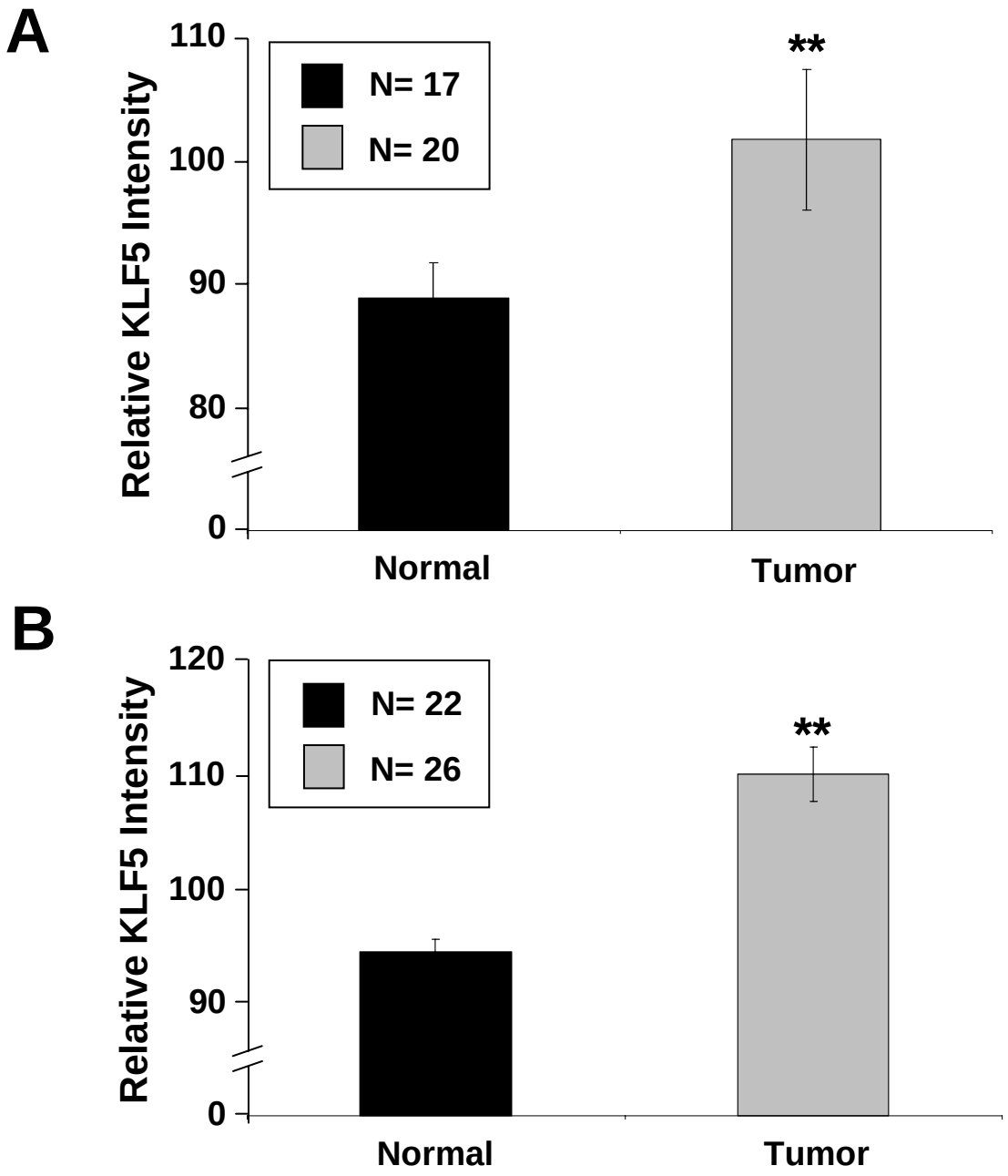
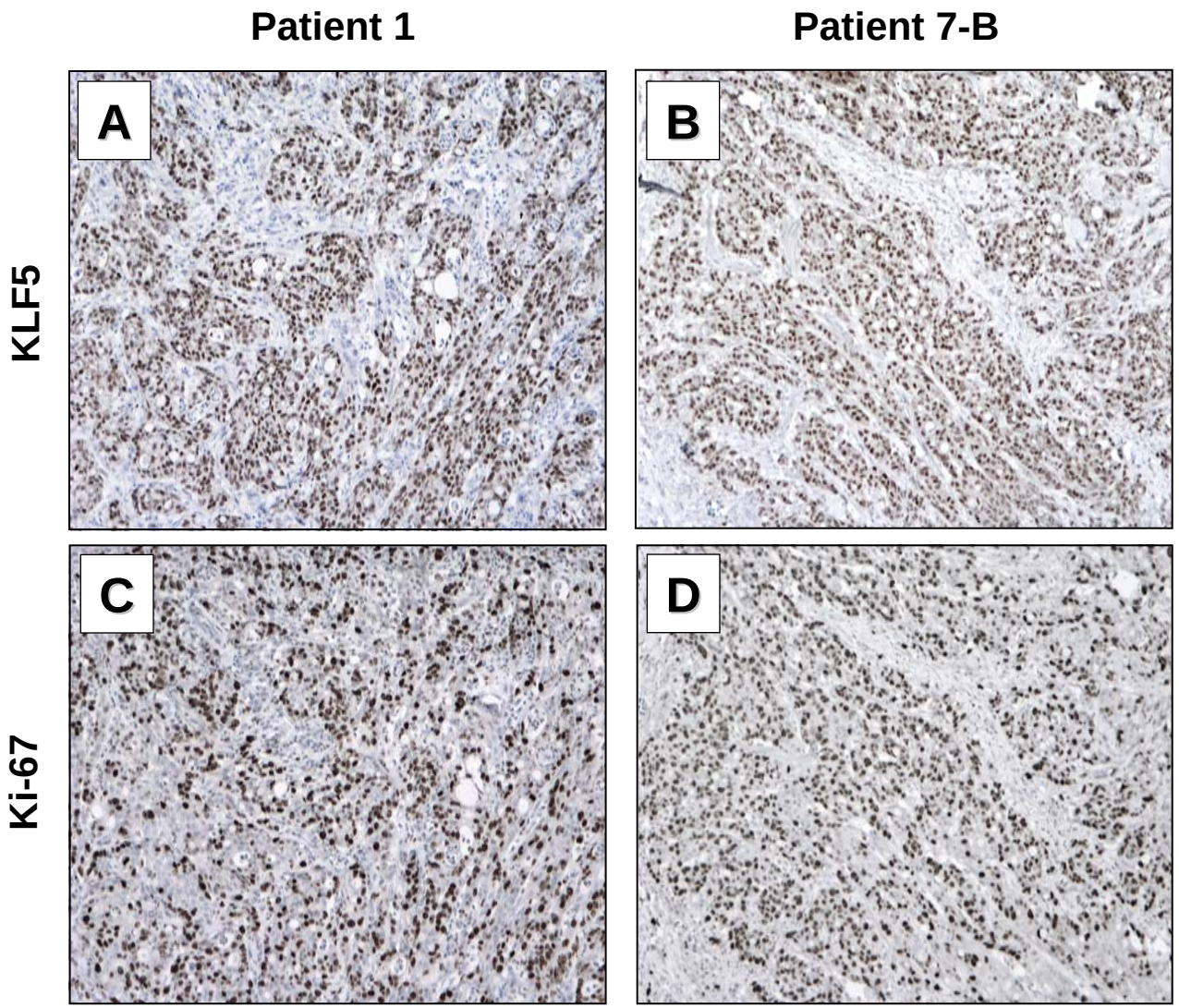


Supplementary Figure 7



Supplementary Figure 8

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. Cell proliferation upon treatment of KLF5 siRNA in uninduced IEC-iKRas cells.

Uninduced IEC-iKRas cells were transfected with KLF5-specific siRNA 3, NS siRNA or left untransfected. Cells were seeded on day 0 onto a 6-well dish at a density of 2 x 10^5 cells/well. Cell proliferation was measured by cell count for a period of 3 days post-transfection. $N = 6$.

Supplementary Figure 2. Quantification of KLF5 protein levels in human colon cancer cell lines.

Densitometric analysis was performed on Western blots of the six human colon cancer cell line lysates for KLF5 and β-actin (Fig. 5A) using the Scion image analysis software. Relative KLF5 levels were calculated by dividing the KLF5 band intensity by that of β-actin.

Supplementary Figure 3. Effects of MEK inhibitors and KLF5 siRNA on proliferation of SW-480 cells.

(A) SW-480 cells were treated with MEK inhibitors, PD98059 or U0126, or DMSO for 24 h. Cell lysates were prepared and analyzed for KLF5, p-ERK, ERK and β-actin by Western blots. (B) SW-480 cells were transfected with KLF5-specific siRNA or NS siRNA, or left untreated for 24 h and lysates analyzed for KLF5, p-ERK, ERK and β-actin. (C) SW-480 cells were treated with MEK inhibitors, DMSO, or left untreated for three days and cell number measured each day. $N = 6$; * $p < 0.05$ comparing MEK inhibitor-treated and DMSO-treated cells. (D) SW-480 cells transfected with KLF5, NS siRNA or left untreated were cultured for three days and cell

numbers measured daily. $N = 6$; * $p < 0.05$ comparing KLF5 siRNA and NS siRNA-transfected cells.

Supplementary Figure 4. Effects of MEK inhibitors and KLF5 siRNA on proliferation of HCT116 cells.

(A) HCT116 cells were treated with MEK inhibitors, PD98059 or U0126, or DMSO for 24 h. Cell lysates were prepared and analyzed for KLF5, p-ERK, ERK and β-actin by Western blots. (B) HCT116 cells were transfected with KLF5-specific siRNA or NS siRNA, or left untreated for 24 h and lysates analyzed for KLF5, p-ERK, ERK and β-actin. (C) HCT116 cells were treated with MEK inhibitors, an equal volume of DMSO, or left untreated for three days and cell number measured each day. $N = 6$; * $p < 0.05$ comparing MEK inhibitor-treated and DMSO-treated cells. (D) HCT116 cells transfected with KLF5, NS siRNA or left untreated were cultured for three days and cell numbers measured daily. $N = 6$; * $p < 0.05$ comparing KLF5 siRNA and NS siRNA-transfected cells.

Supplementary Figure 5. Effects of KLF5 siRNA on proliferation of RKO cells.

RKO cells were transfected with KLF5-specific, NS siRNAs or left untransfected. These cells were then cultured for up to three days and cell number counted daily. $N = 6$.

Supplementary Figure 6. Colony formation assays in colorectal cancer cells over-expressing KLF5. DLD-1and HCT116 cells were transfected with pBKCMV-KLF5 plasmid, which expressed KLF5 protein constitutively [2] or with pBKCMV empty vector control. Transfected cells were then seeded onto 6-well dishes at a density of 1 x 10^4 cells/well and

maintained in appropriate media with neomycin selection for 2 weeks, with fresh media added every 3 days. The cells were then fixed and stained with methylene blue solution containing methanol. Appearance of colonies on the plate represents the degree of transformation in the cells. (A and B) Representative culture dishes of DLD-1 and HCT116 cells transfected with the pBKCMV empty vector. (C and D) Representative culture dishes of DLD-1 and HCT116 cells transfected with pBKCMV-KLF5 vector. $N = 9$ in each experiment.

Supplementary Figure 7. Correlation between KLF5 and Ki67 staining in tumors with mutated KRAS.

(A and B) Representative colon tumor sections from patients 1 and 7 were assessed for KLF5 levels by immunohistochemistry. Nuclei are counterstained with Mayer's hematoxylin. (C and D) Adjacent sections from panels A and B stained for Ki67, a proliferation marker.

Supplementary Figure 8. Quantification of KLF5 staining intensities in normal and tumor specimens.

The intensity of KLF5 staining per cell was quantified as described in Supplementary Methods. Relative KLF5 intensity, for both normal and tumor samples, was calculated by averaging KLF5 intensity per cell in the area of positive KLF5 staining. (A) Relative KLF5 intensity for normal and tumor intestinal tissues obtained from villin-KRAS^{V12G} transgenic. (B) Relative KLF5 intensity for matched normal colon and tumor tissues obtained from colon cancer patients. ** $p < 0.001$.