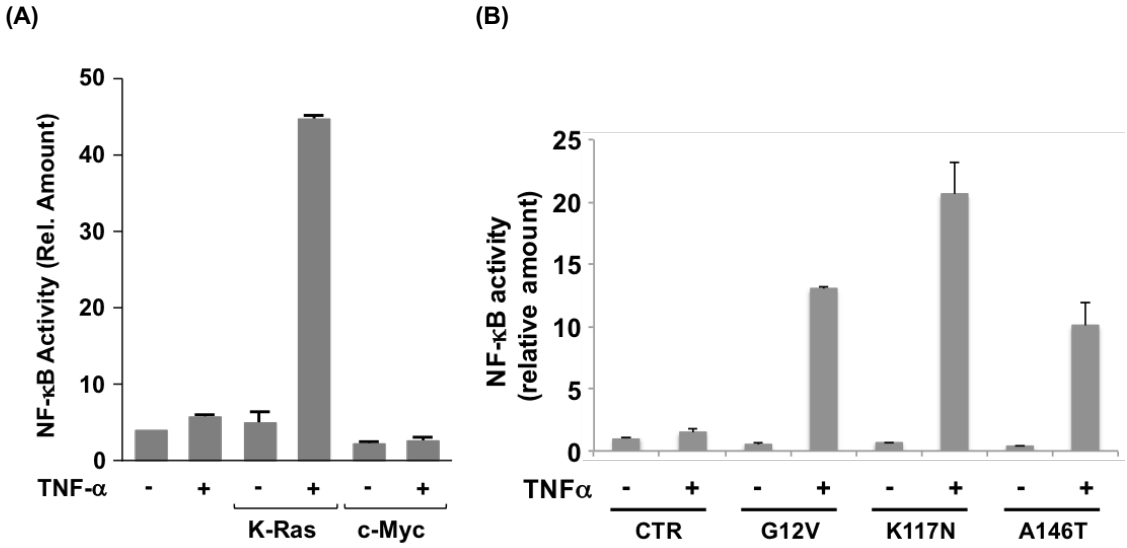
Supplementary Figures

For:

Oncogenic Ras mutant causes the hyper-activation of NF-κB via acceleration of its transcriptional activation.

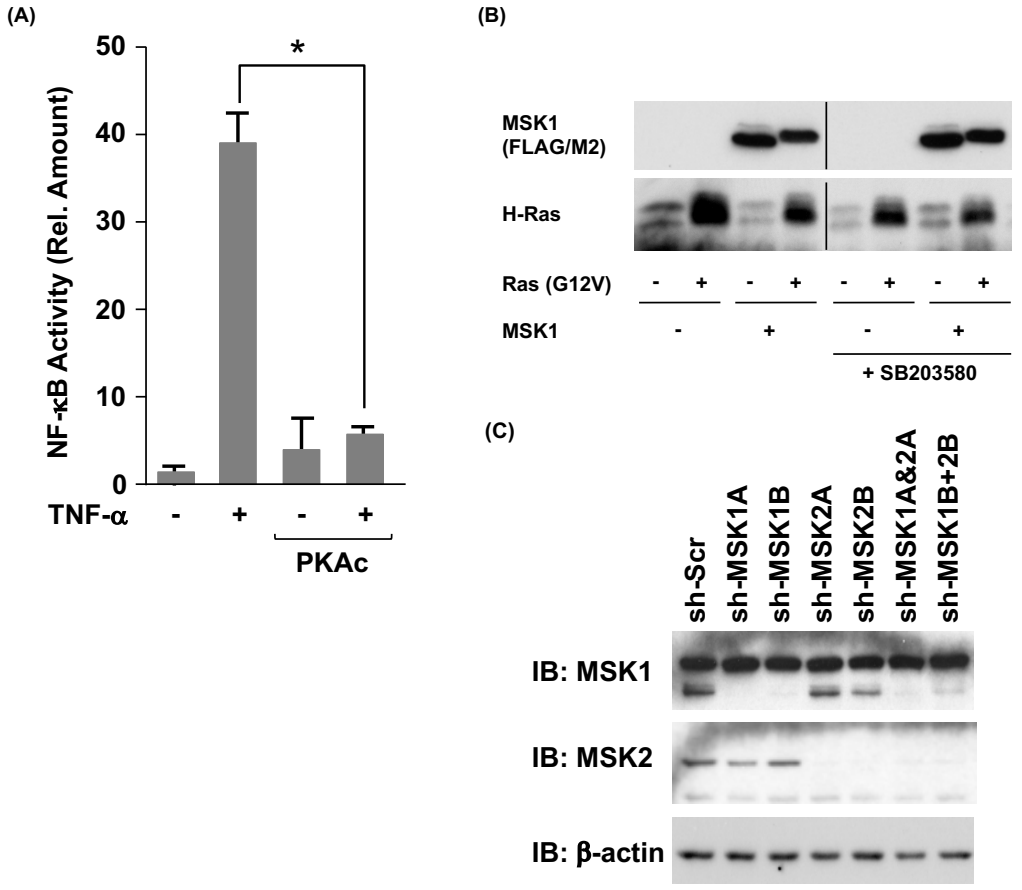
Kenji Tago[1, *], Megumi Funakoshi-Tago[2], Satoshi Ohta[1], Hirotoshi Kawata[3], Hiroshi Saitoh[1], Hisanaga Horie[4], Chihiro Aoki-Ohmura[1], Junji Yamauchi[5], Akira Tanaka[3], Jitsuhiro Matsugi[1] and Ken Yanagisawa[1]

[1] Division of Structural Biochemistry, Department of Biochemistry, [3]Department of Pathology, [4]Department of Surgery, Jichi Medical University, 3311-1 Yakushiji, Shimotsuke, Tochigi, Japan, 321-0498, [2] Division of Hygienic Chemistry, Faculty of Pharmacy, Keio University, 1-5-30 Shibakoen, Minato-ku, Tokyo 105-8512, Japan, [5] Laboratory of Molecular Neuroscience and Neurology, Tokyo University of Pharmacy and Life Sciences, Hachioji, Tokyo, 192-0392, Japan



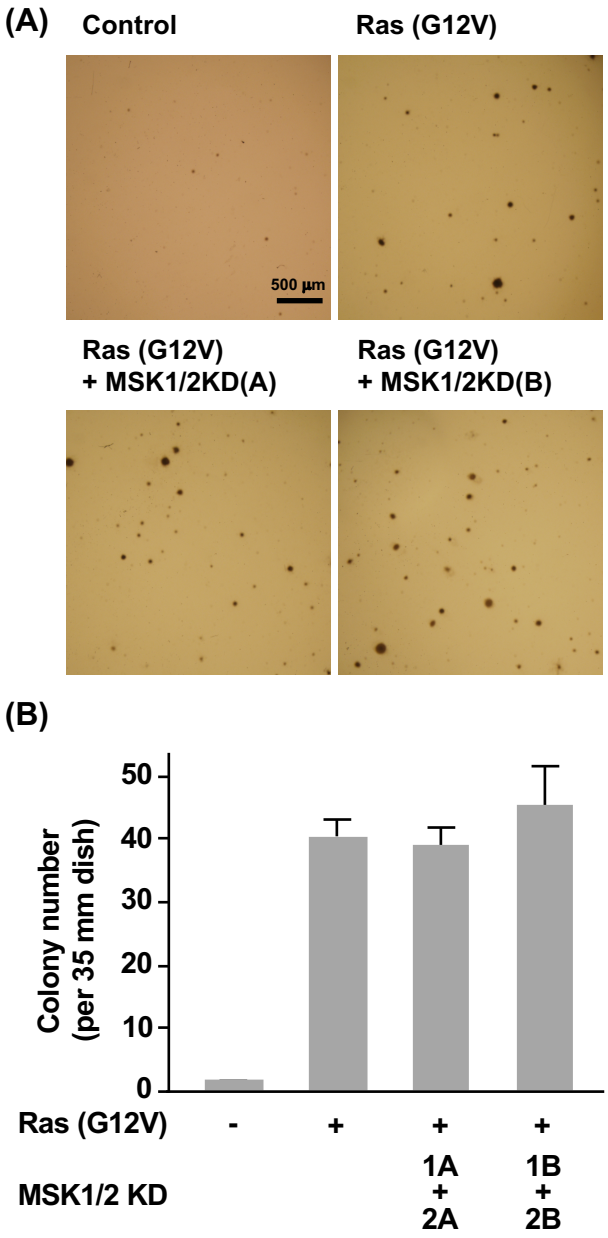
Supplementary Figure 1

(A) KF-8 cells were infected with control retroviruses or retroviruses harboring K-Ras (G12V) or c-Myc. After puromycin selection, cells were stimulated with 10 μg/ml TNFα for indicated periods. Then, cells were lysed, and the luciferase assay was performed. (B) Using KF-8 cells infected with control retroviruses or retroviruses harboring K-Ras mutants including G12V, K117N and A146T, the luciferase assay was performed as shown in (A). In the graph, error bars = S.D. (n=3, *P<0.005).



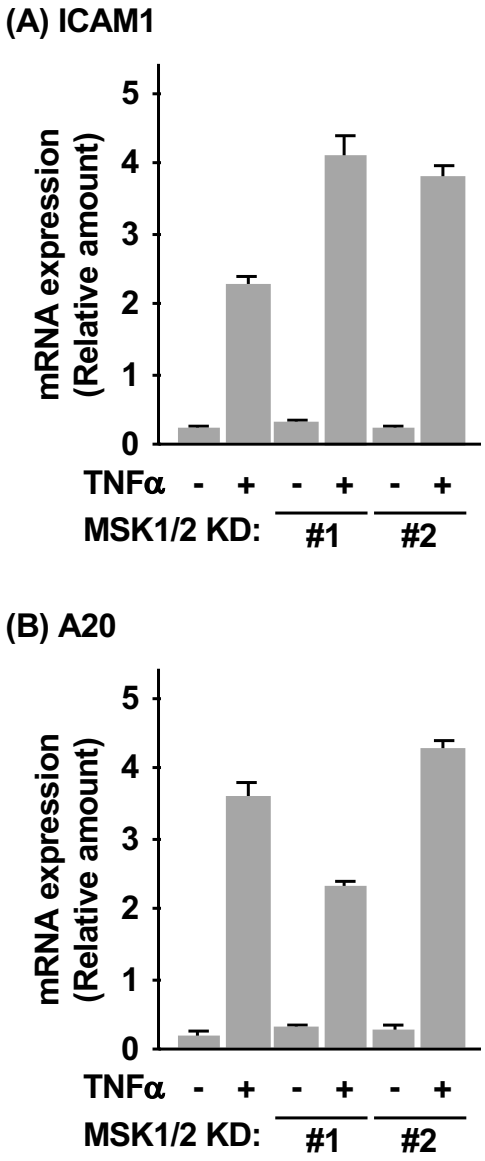
Supplementary Figure 2

(A) HEK293T cells were transfected with a luciferase vector including κB-responsive element with/without FLAG-PKAc, a catalytic subunit of PKA. Then, using the transfected cells, luciferase assays were performed. In graph, error bars indicate standard deviation (S.D., n=3), and the results of calculations of independent t-tests are shown (*P<0.005). (B) The expression of FLAG-MSK1 and H-Ras (G12V) in cells analyzed for *in vitro* kinase assay (Figure 5A) was analyzed by immunoblotting analysis using antibodies against FLAG-tag (M2) and H-Ras. (C) KF-8 cells were infected with retroviruses harboring scrambled short-hairpin RNA, sh-MSK1 (1A or 1B) or sh-MSK2 (2A or 2B). After puromycin selection, cells were lysed, and immunoblotting analysis was performed to evaluate the knockdown efficiency of MSK1 and MSK2. β-actin was used as a loading control.



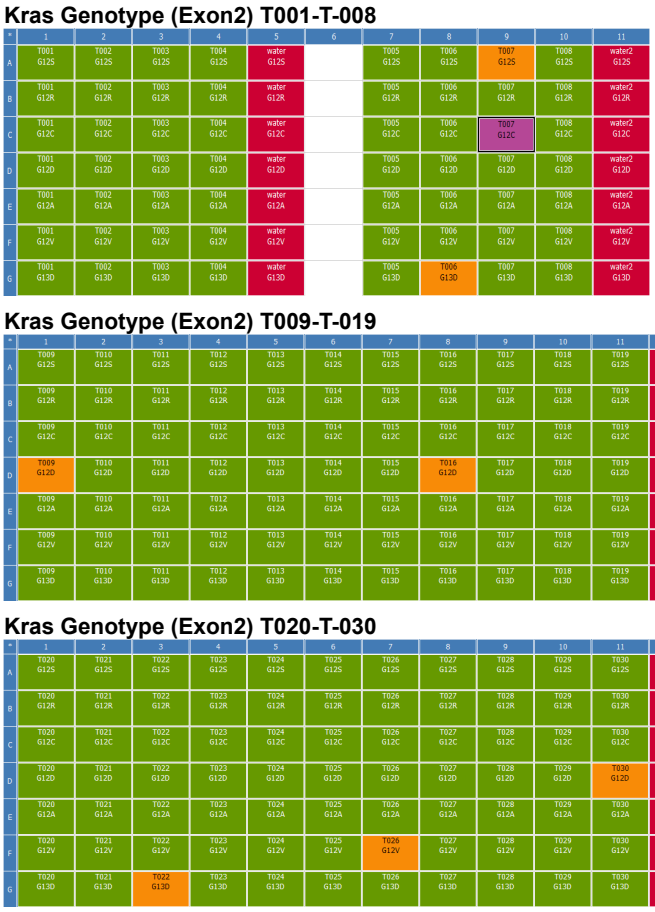
Supplementary Figure 3

(A) NIH-3T3 cells were infected with indicated retroviruses as described in Figure 5B. After puromycin selection, cells (1×10^4 cells) were seeded on soft agar media in 35 mm dishes. After 3 weeks, the number of colonies were counted and shown in graph as shown in (B). In the graph, error bars = S.D. (n=3, **P*<0.005). In the photograph, 500 μm scale bar was shown.



Supplementary Figure 4

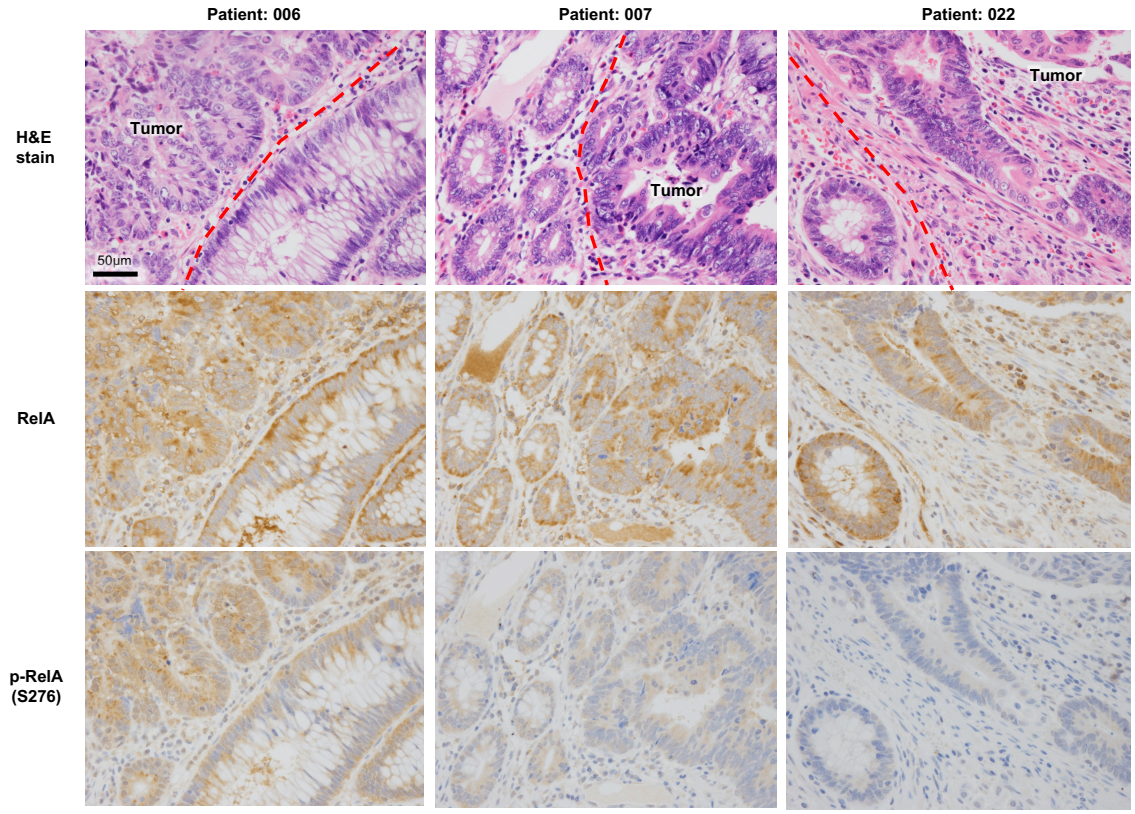
SW620, a colorectal cancer-derived cell line was transfected with siRNAs against both MSK1 and MSK2. Forty-eight hr later, cells were stimulated with/without 10 ng/mL TNFα for 3 h, and then total RNAs were extracted from these cells. The expressions of NF-κB target genes, ICAM1 and A20 were analyzed by quantitative RT-PCR, and results were shown in the graphs (A) and (B). In the graph, error bars = S.D. (n=3, **P*<0.005).



Patient Number	KRas mutation
006	G13D
007	G12S/G12C
009	G12D
016	G12D
022	G13D
026	G12V
030	G12D

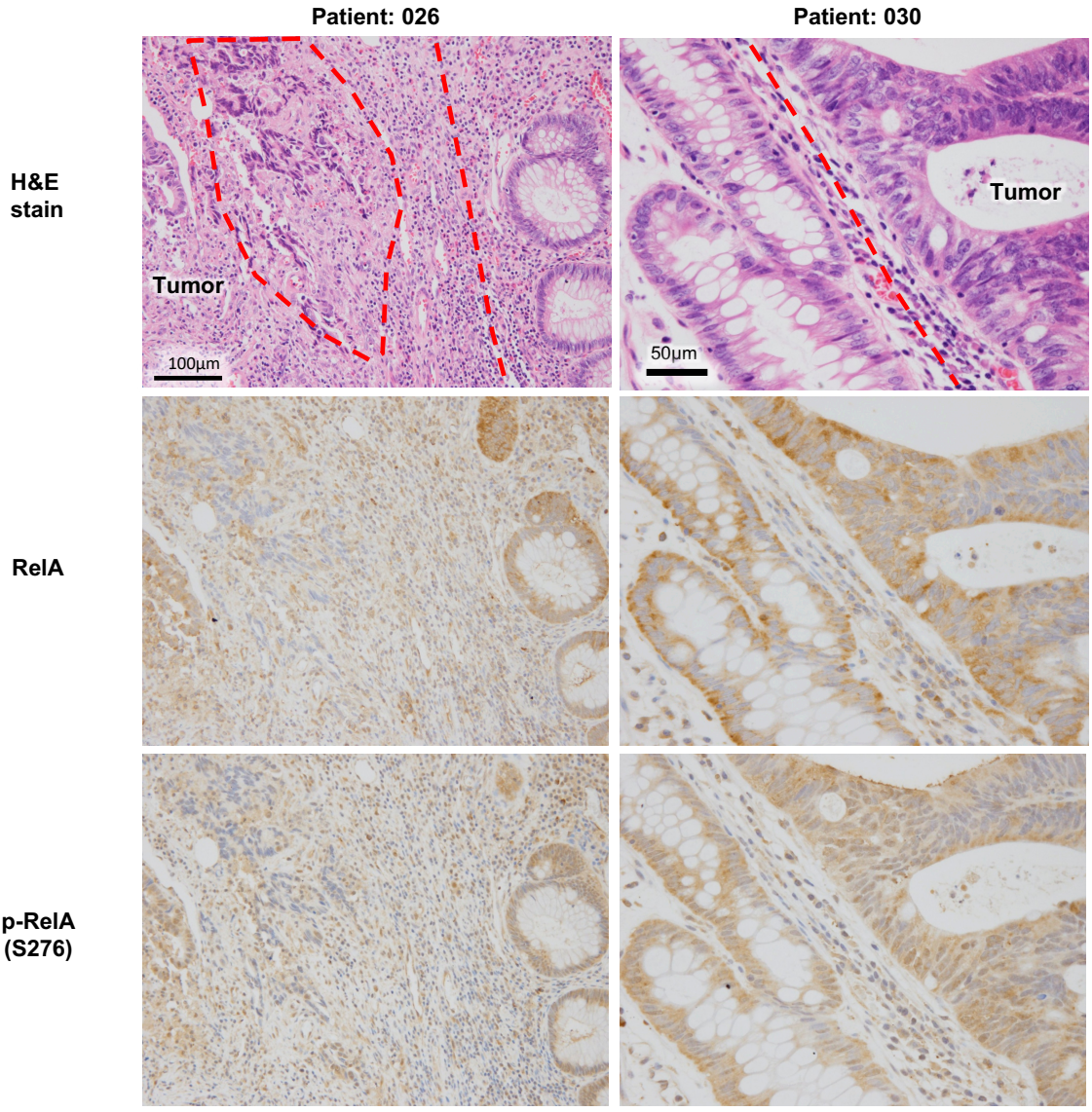
Supplementary Figure 5

Genomic DNA was extracted from tumor tissues of 30 colorectal cancer patients (designated as T001 to T030). To detect K-Ras mutations such as G12S, G12R, G12C, G12D, G12A, G12V, and G13D, quantitative PCR was performed. Detected K-Ras mutations are colored purple (homozygous) or yellow (heterozygous). As result, it was concluded that 7 colorectal cancer patients (006, 007, 009, 016, 022, 026, and 030) harbored K-Ras mutations in exon 2 of the K-Ras gene.



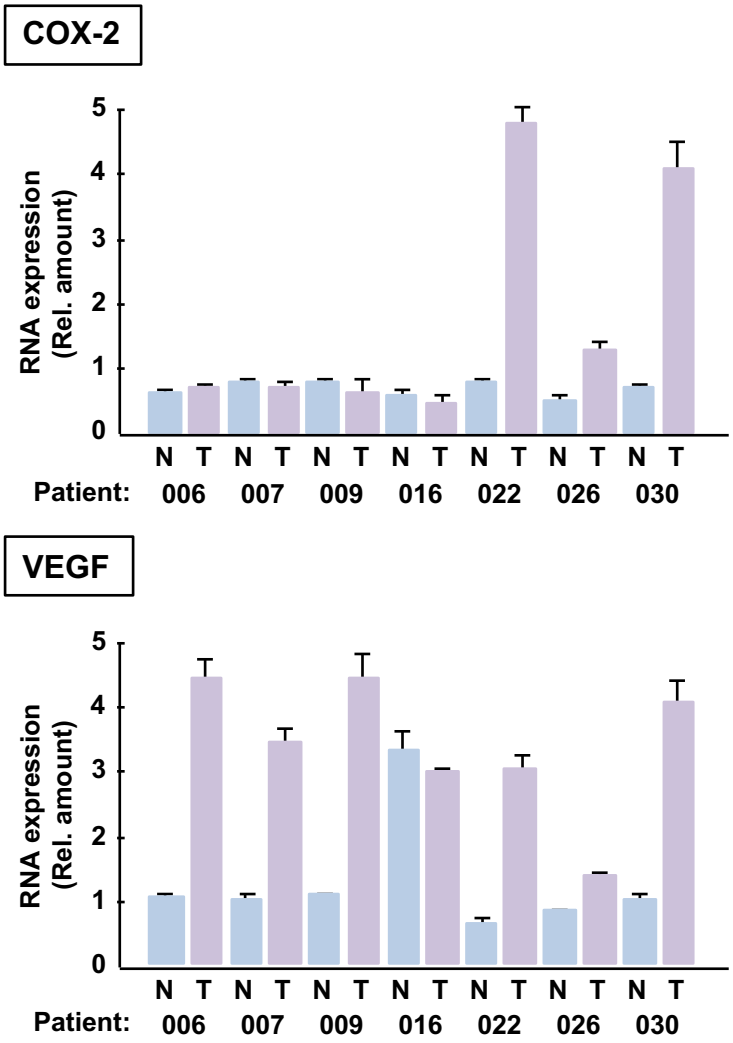
Supplementary Figure 6

Utilizing paired samples of tumor and normal tissues from K-Ras (+) patients, immunohistochemical analyses were performed to detect total p65/RelA and phosphorylated p65/RelA (Ser276). H & E staining is also shown to distinguish tumor and normal tissues. Among 7 patients, 2 samples (009 and 016) were shown in Figure 7A. In the photograph, 50 μm scale bar was shown excepting patient 026 including 100 μm scale bar.



Supplementary Figure 6

(Continued)



Supplementary Figure 7

Total RNA was extracted from normal and tumor tissues from each patient harboring K-Ras mutations. Then, quantitative RT-PCR was performed to evaluated the mRNA expression of COX-2 and VEGF. In the graph, error bars = S.D. (n=3).