

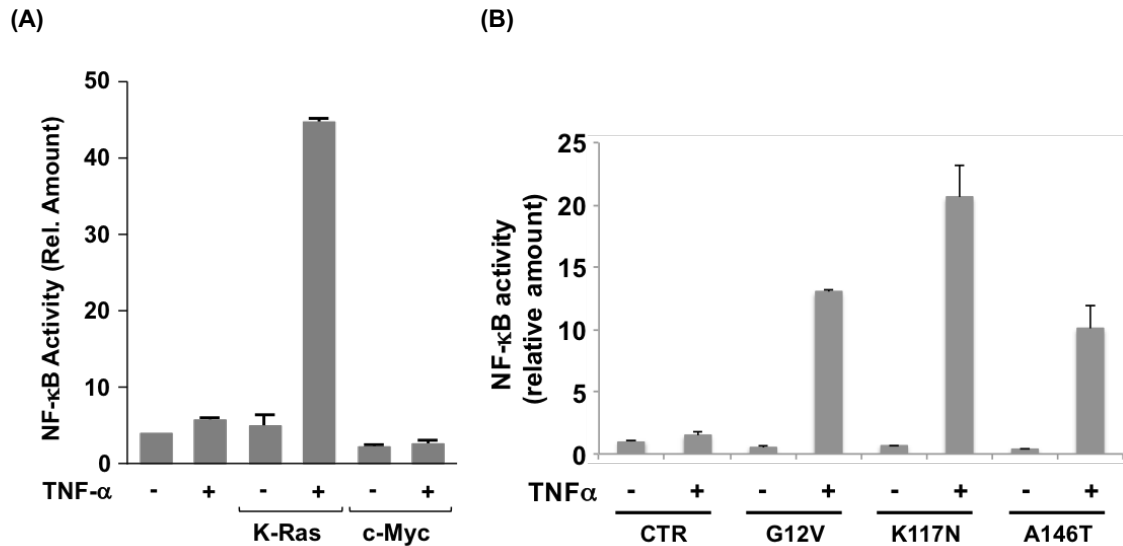
Supplementary Figures

For:

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

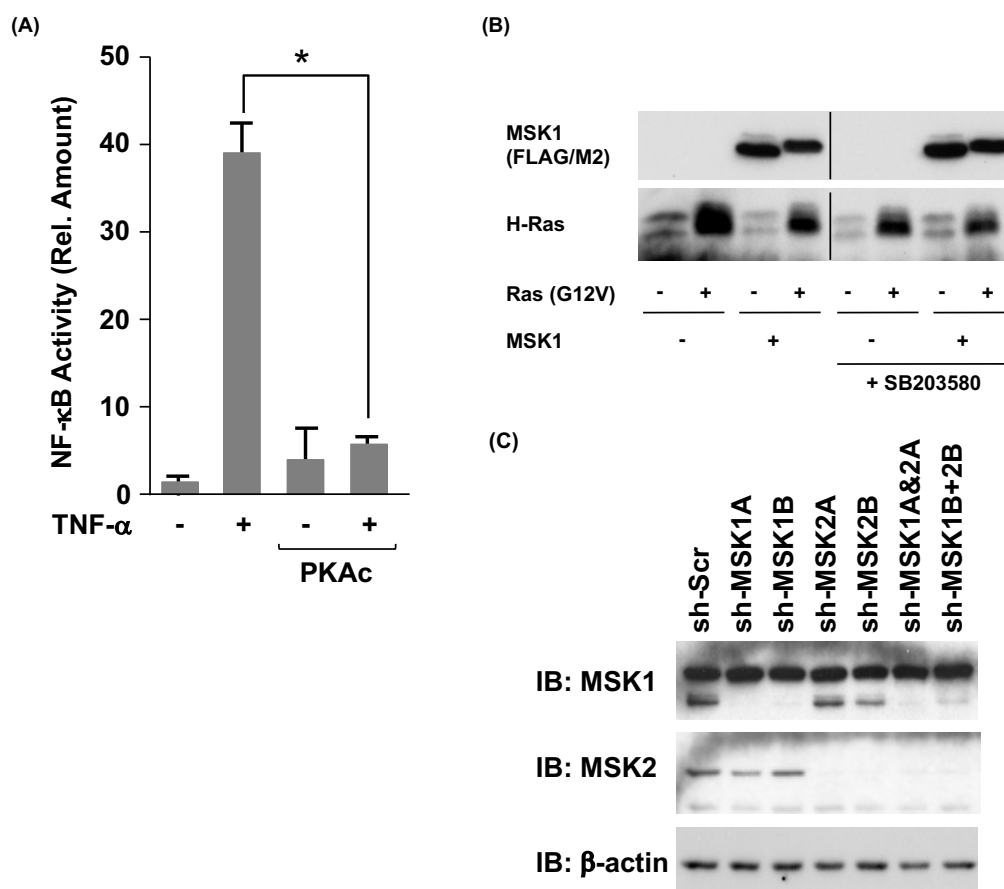
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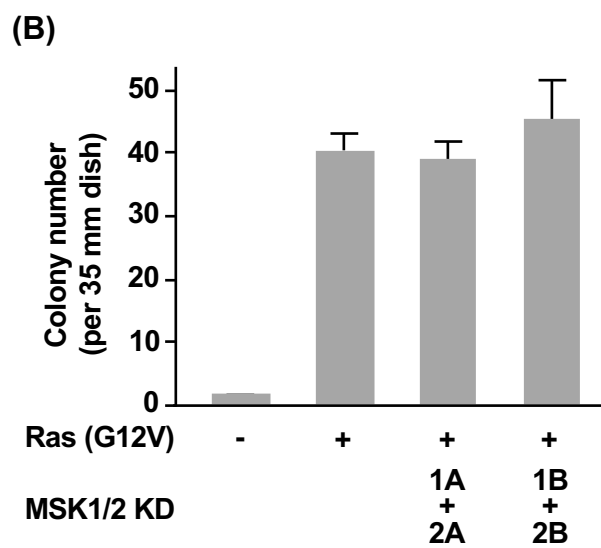
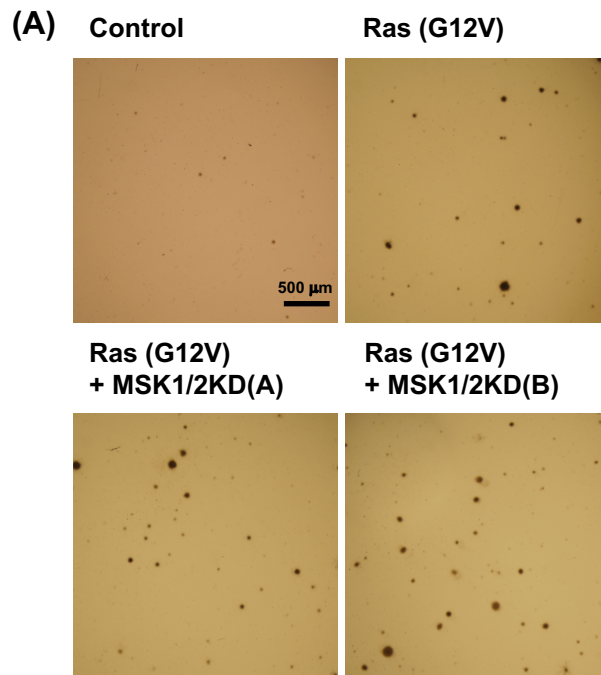
Supplementary Figure 1

(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 μ g/ml TNF α 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

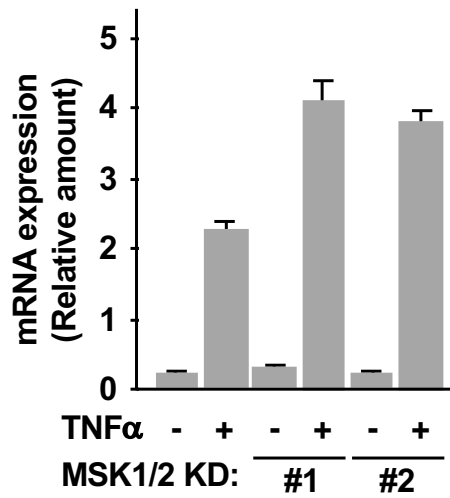
(A) HEK293T cells were transfected with a luciferase vector including κ B-responsive element with/without FLAG-PKAc, a catalytic subunit of PKA. Then, using the transfected cells, luciferase assays were performed. In graph, error bars indicate standard deviation (S.D., n=3), and the results of calculations 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.



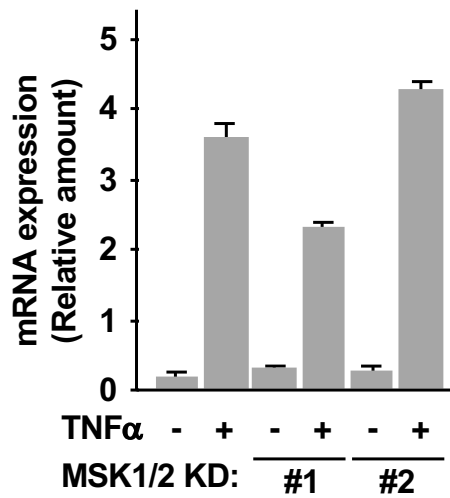
Supplementary Figure 3

(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.

(A) ICAM1



(B) A20



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 TNF α for 3 h, and then total RNAs were extracted from these cells. The expressions of NF- κ B 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).

Kras Genotype (Exon2) T001-T-008

	1	2	3	4	5	6	7	8	9	10	11
A	T001 G12S	T002 G12S	T003 G12S	T004 G12S	water G12S		T005 G12S	T006 G12S	T007 G12S	T008 G12S	water2 G12S
B	T001 G12R	T002 G12R	T003 G12R	T004 G12R	water G12R		T005 G12R	T006 G12R	T007 G12R	T008 G12R	water2 G12R
C	T001 G12C	T002 G12C	T003 G12C	T004 G12C	water G12C		T005 G12C	T006 G12C	T007 G12C	T008 G12C	water2 G12C
D	T001 G12D	T002 G12D	T003 G12D	T004 G12D	water G12D		T005 G12D	T006 G12D	T007 G12D	T008 G12D	water2 G12D
E	T001 G12A	T002 G12A	T003 G12A	T004 G12A	water G12A		T005 G12A	T006 G12A	T007 G12A	T008 G12A	water2 G12A
F	T001 G12V	T002 G12V	T003 G12V	T004 G12V	water G12V		T005 G12V	T006 G12V	T007 G12V	T008 G12V	water2 G12V
G	T001 G13D	T002 G13D	T003 G13D	T004 G13D	water G13D		T005 G13D	T006 G13D	T007 G13D	T008 G13D	water2 G13D

Kras Genotype (Exon2) T009-T-019

	1	2	3	4	5	6	7	8	9	10	11	12
A	T009 G12S	T010 G12S	T011 G12S	T012 G12S	T013 G12S	T014 G12S	T015 G12S	T016 G12S	T017 G12S	T018 G12S	T019 G12S	Negative CTR G12S
B	T009 G12R	T010 G12R	T011 G12R	T012 G12R	T013 G12R	T014 G12R	T015 G12R	T016 G12R	T017 G12R	T018 G12R	T019 G12R	Negative CTR G12R
C	T009 G12C	T010 G12C	T011 G12C	T012 G12C	T013 G12C	T014 G12C	T015 G12C	T016 G12C	T017 G12C	T018 G12C	T019 G12C	Negative CTR G12C
D	T009 G12D	T010 G12D	T011 G12D	T012 G12D	T013 G12D	T014 G12D	T015 G12D	T016 G12D	T017 G12D	T018 G12D	T019 G12D	Negative CTR G12D
E	T009 G12A	T010 G12A	T011 G12A	T012 G12A	T013 G12A	T014 G12A	T015 G12A	T016 G12A	T017 G12A	T018 G12A	T019 G12A	Negative CTR G12A
F	T009 G12V	T010 G12V	T011 G12V	T012 G12V	T013 G12V	T014 G12V	T015 G12V	T016 G12V	T017 G12V	T018 G12V	T019 G12V	Negative CTR G12V
G	T009 G13D	T010 G13D	T011 G13D	T012 G13D	T013 G13D	T014 G13D	T015 G13D	T016 G13D	T017 G13D	T018 G13D	T019 G13D	Negative CTR G13D

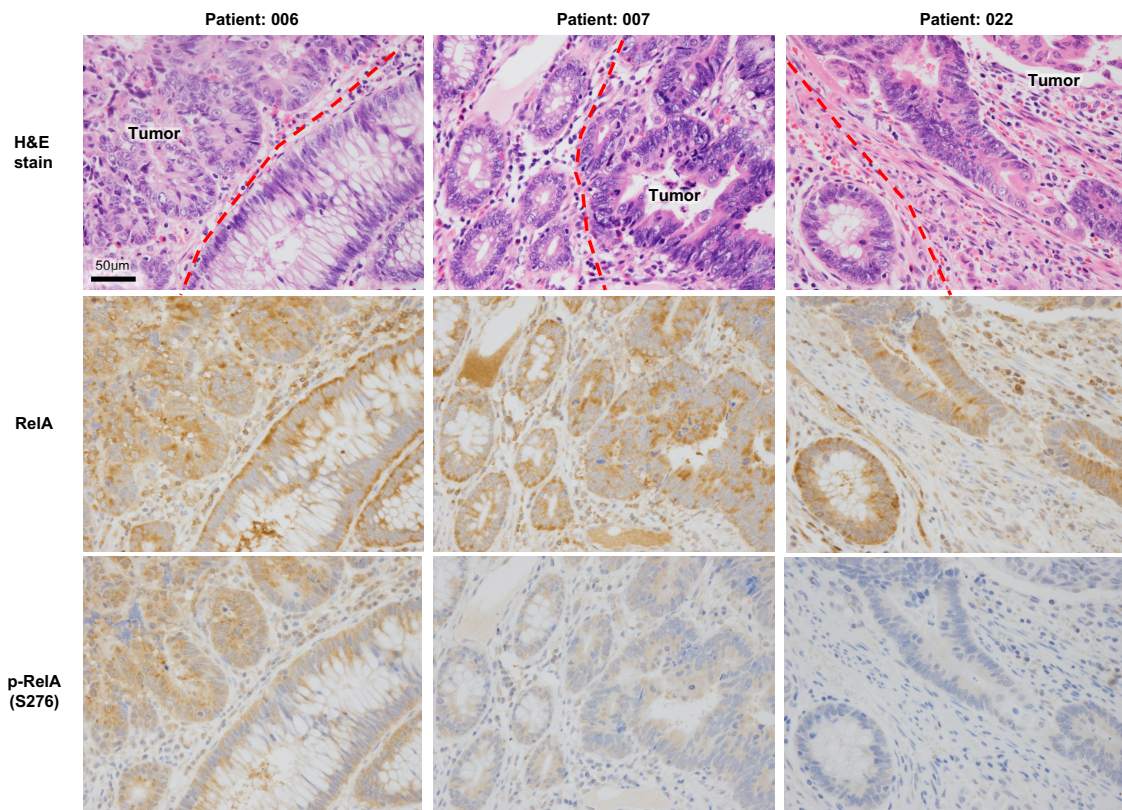
Kras Genotype (Exon2) T020-T-030

	1	2	3	4	5	6	7	8	9	10	11	12
A	T020 G12S	T021 G12S	T022 G12S	T023 G12S	T024 G12S	T025 G12S	T026 G12S	T027 G12S	T028 G12S	T029 G12S	T030 G12S	Water G12S
B	T020 G12R	T021 G12R	T022 G12R	T023 G12R	T024 G12R	T025 G12R	T026 G12R	T027 G12R	T028 G12R	T029 G12R	T030 G12R	Water G12R
C	T020 G12C	T021 G12C	T022 G12C	T023 G12C	T024 G12C	T025 G12C	T026 G12C	T027 G12C	T028 G12C	T029 G12C	T030 G12C	Water G12C
D	T020 G12D	T021 G12D	T022 G12D	T023 G12D	T024 G12D	T025 G12D	T026 G12D	T027 G12D	T028 G12D	T029 G12D	T030 G12D	Water G12D
E	T020 G12A	T021 G12A	T022 G12A	T023 G12A	T024 G12A	T025 G12A	T026 G12A	T027 G12A	T028 G12A	T029 G12A	T030 G12A	Water G12A
F	T020 G12V	T021 G12V	T022 G12V	T023 G12V	T024 G12V	T025 G12V	T026 G12V	T027 G12V	T028 G12V	T029 G12V	T030 G12V	Water G12V
G	T020 G13D	T021 G13D	T022 G13D	T023 G13D	T024 G13D	T025 G13D	T026 G13D	T027 G13D	T028 G13D	T029 G13D	T030 G13D	Water G13D

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

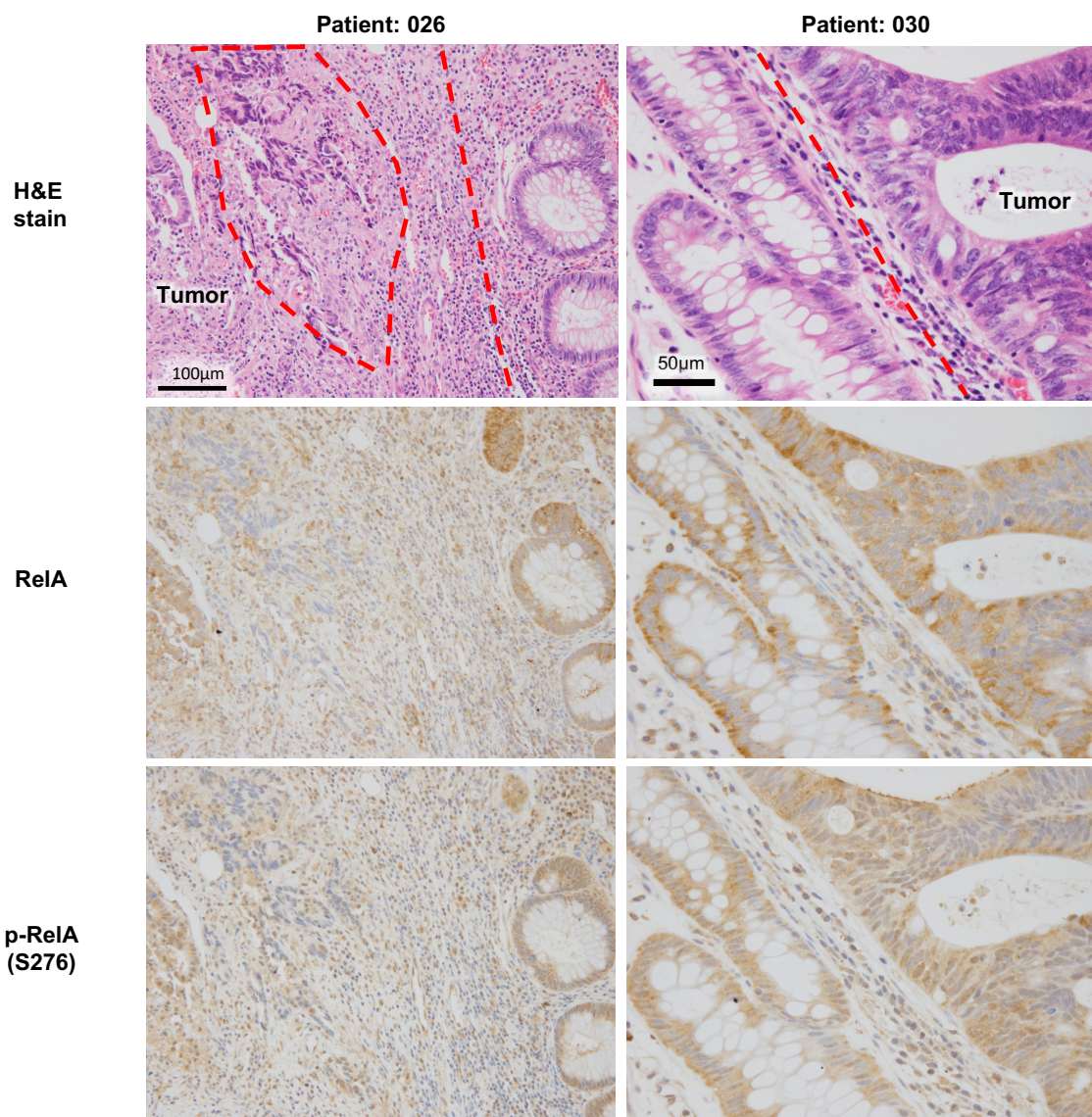
Supplementary Figure 5

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

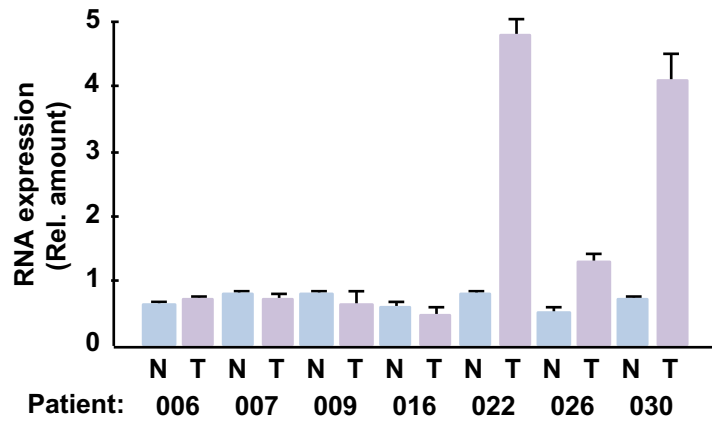
Utilizing paired samples of tumor and normal tissues from K-Ras (+) patients, immunohistochemical analyses were performed to detect total p65/RelA and phosphorylated p65/RelA (Ser276). H & E staining is also shown to distinguish tumor and normal tissues. Among 7 patients, 2 samples (009 and 016) were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



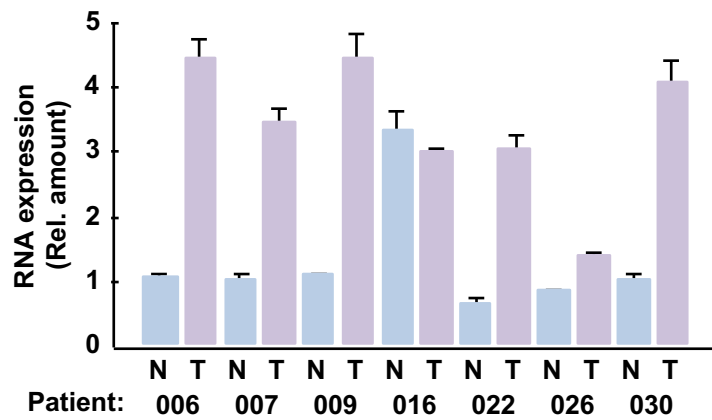
Supplementary Figure 6

(Continued)

COX-2



VEGF



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 evaluate the mRNA expression of COX-2 and VEGF. In the graph, error bars = S.D. (n=3).