

Figure S1: IHC evaluation of GPNMB.

To determine the appropriate IHC conditions, we created cell blocks containing three cell lines (GPNMB expression: high, SK-BR-3; moderate, MKN1; and low, LS174T; similar to Fig. 1) and used PROTEASE 1 for antigen retrieval. These results were consistent with western blot results (Fig. 1) and reverse transcription polymerase chain reaction [19]. Normal goat IgG was used as a negative control (NC).

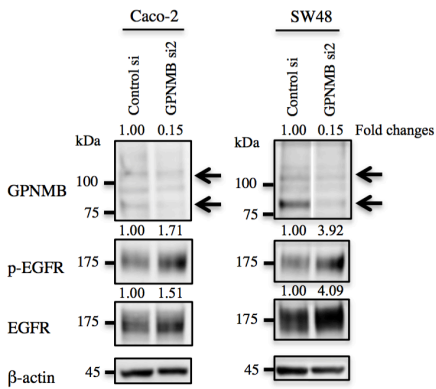
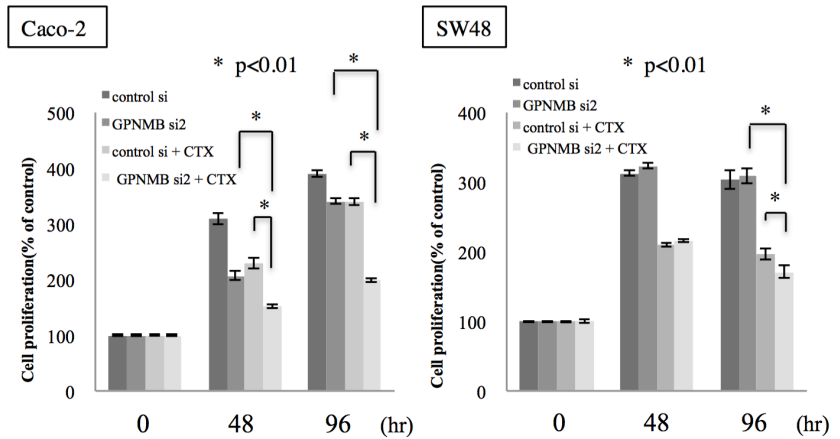
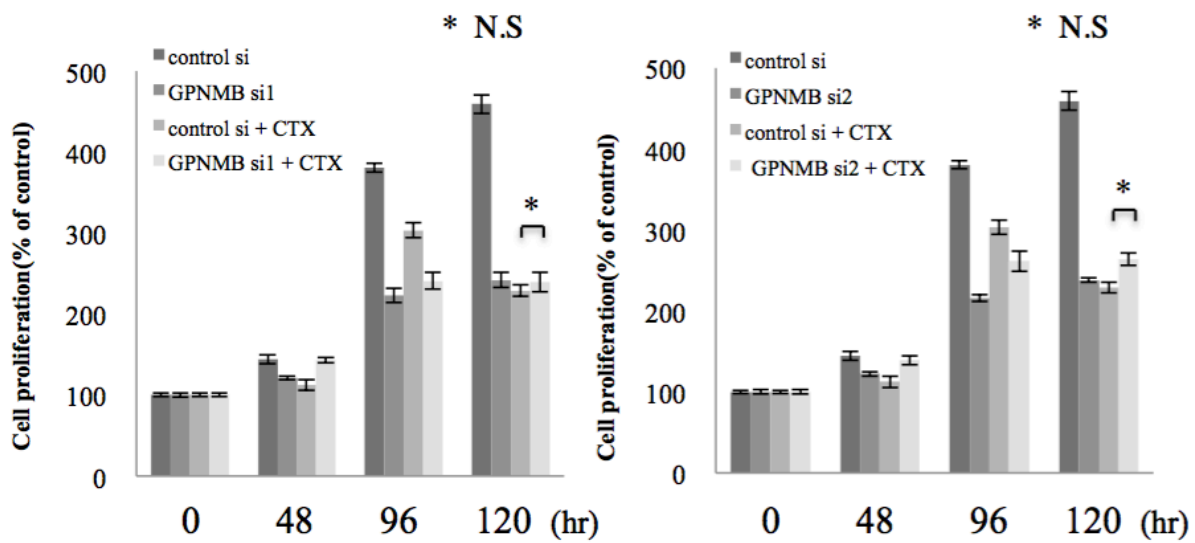
A**B**

Figure S2: Cell-proliferation assay following GPNMB knockdown using different siRNAs.

A. Two CRC cell lines (Caco-2 and SW48) were treated with GPNMBsi2 and Controlsi, and GPNMB, EGFR, and p-EGFR expression levels were evaluated. Relative ratios of protein expression are indicated, respectively. **B.** Cells were treated with cetuximab (100 μ g/mL) after GPNMB depletion, followed by measurement of cell growth by MTT assay. The synergistic effect of growth suppression was determined to be similar to that elicited by use of GPNMBsi1 (Fig. 5).

LS174T



SW1417

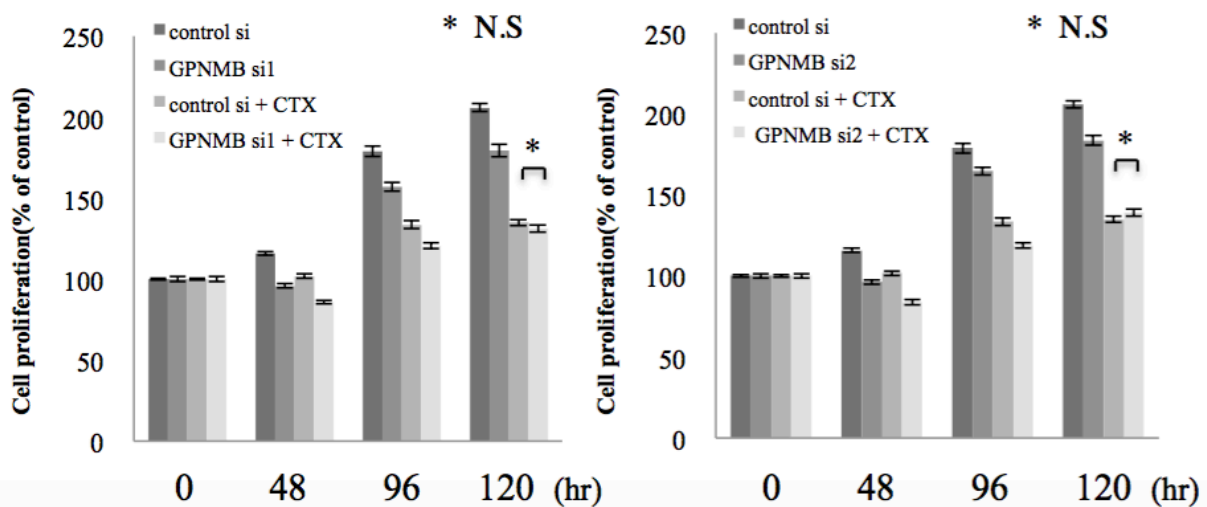


Figure S3. Cell-proliferation assay.

KRAS-mutant (LS174T) or *BRAF*-mutant (SW1417) CRC cell lines were treated with CTX after GPNMB depletion, followed by measurement of cell growth by MTT assay. Both cell lines showed no synergistic effect following the combined use of GPNMBsi1/2 and CTX treatment.

Table S1: Clinical features in seven CRC patients with liver metastasis treated with chemotherapy and anti-EGFR therapy.

Case	Age	Sex	Location	Operation for primary lesion	<i>KRAS</i> status	Regimen of chemotherapy	Response	Outcome
1	64	F	D	Left colectomy	wild	FOLFOX+Cmab 3 cycles FOLFOX 2 cycles	PR	Alive
2	57	F	S	Sigmoidectomy	wild	FOLFOX+Pmab 6 cycles	PD	Dead
3	59	M	Rs	Low anterior resection	wild	FOLFOX+Pmab 6 cycles	PR	Alive
4	57	F	S	Hartmann operation	wild	FOLFOX+Cmab 7 cycles	PR	Alive
5	47	M	S	Sigmoidectomy	wild	FOLFOX+Cmab 8 cycles	PR	Alive
6	66	M	Rs	High anterior resection	wild	FOLFOX+Cmab 8 cycles	PR	Alive
7	73	F	D	Sigmoidectomy	wild	FOLFOX+Pmab 6 cycles	PR	Alive

Seven CRC patients with metachronous liver metastasis after treatment with chemotherapy and anti-EGFR therapy are shown. All patients underwent hepatectomy or segmentectomy. Six patients showed PR, however, one patient (Case 2) resulted in PD.

Cmab: Cetuximab; D: Descending colon; F: Female; FOLFOX: Folinic acid, fluorouracil, and oxaliplatin; M: Male; PD: Progressive disease; Pmab: Pertuzumab; PR: Partial response; Rs: Rectosigmoid colon; S: Sigmoid colon.