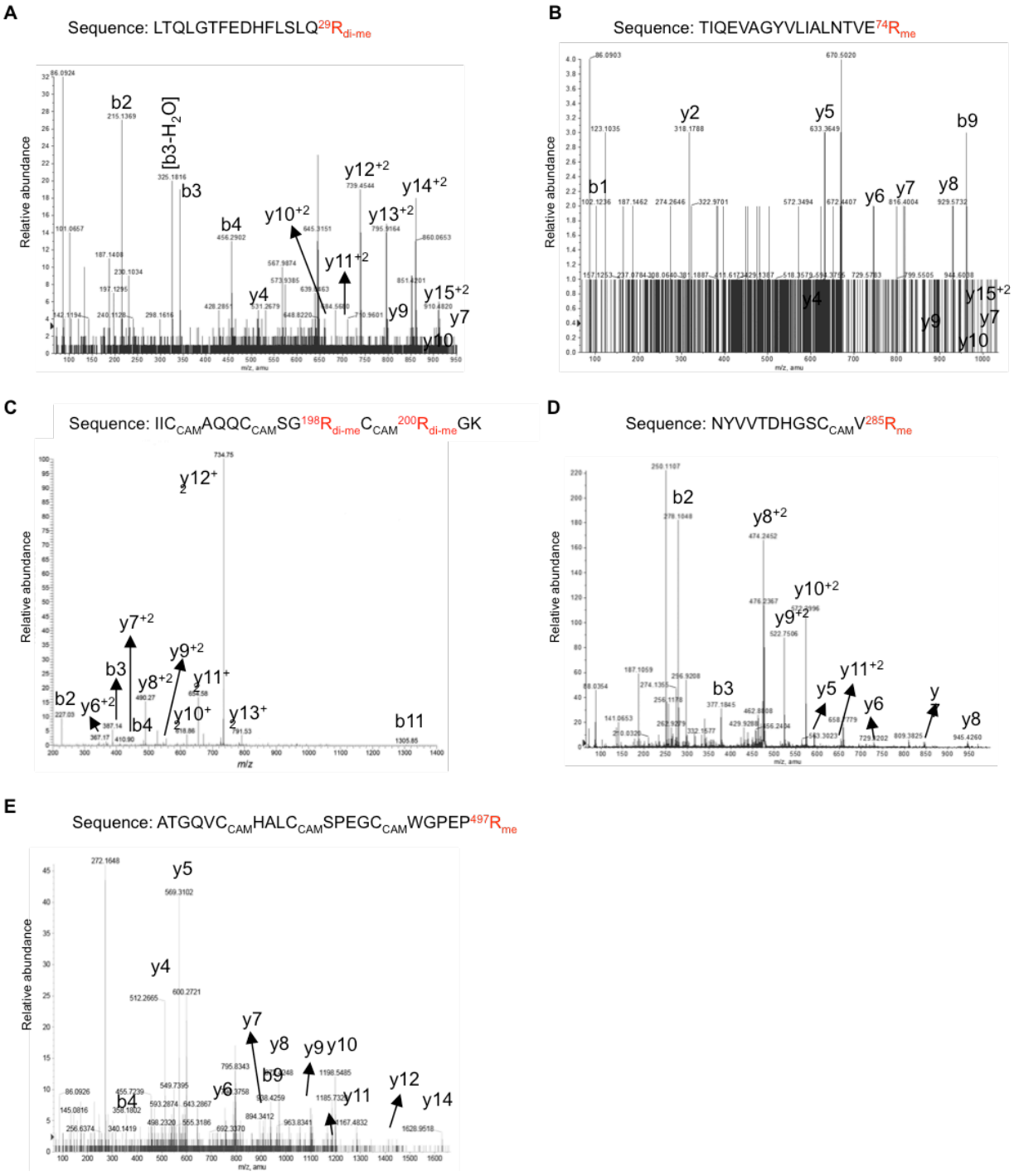
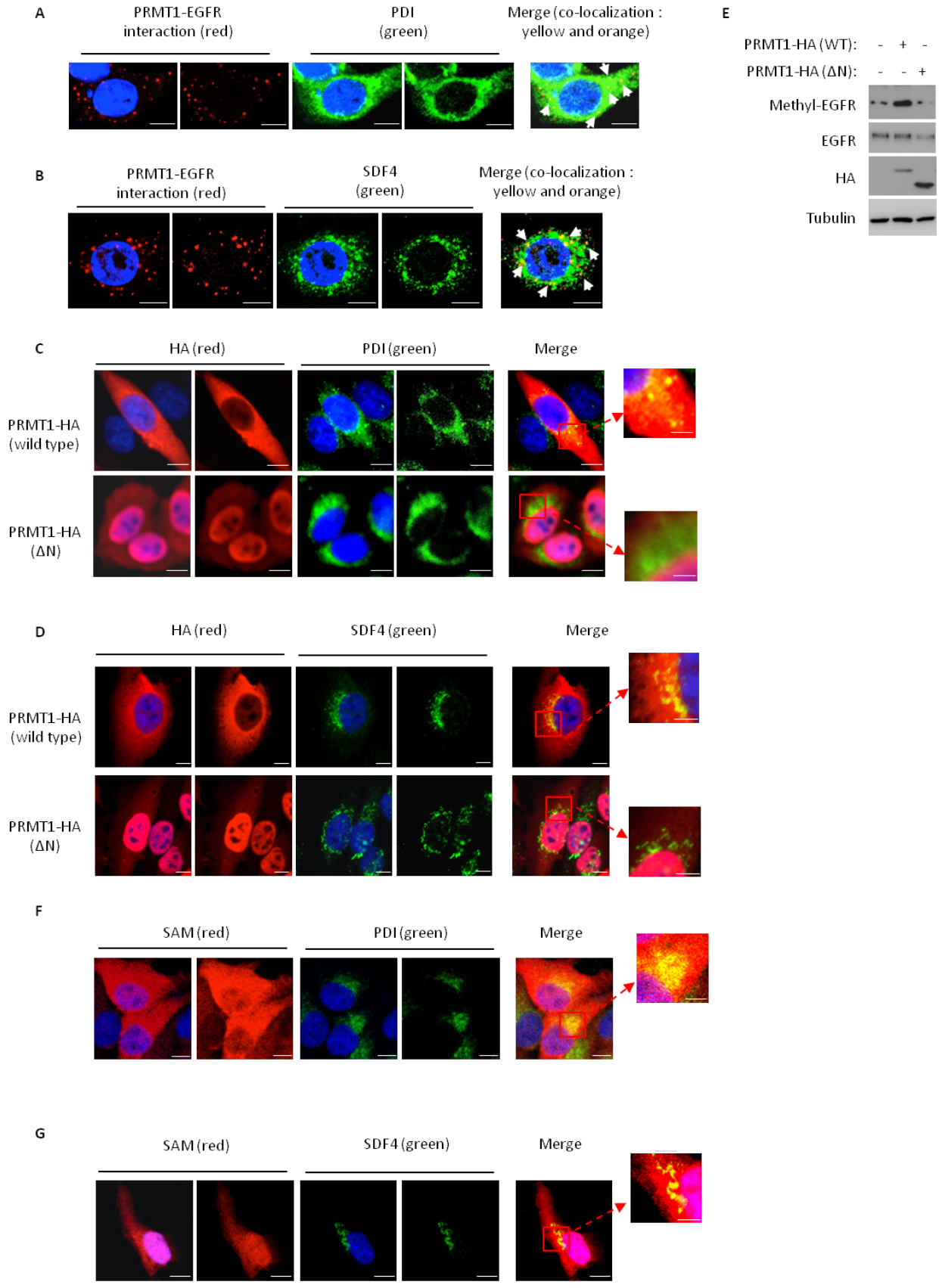
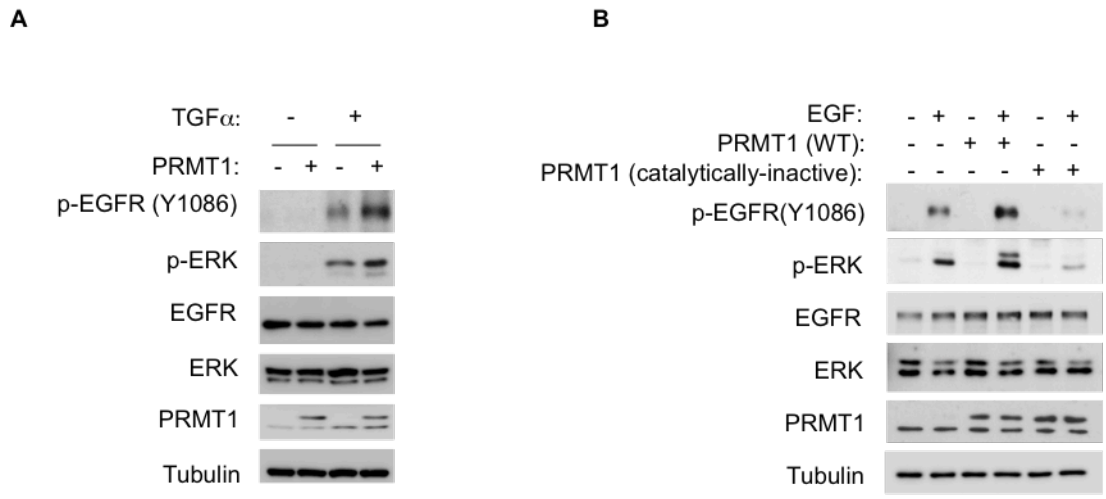
Supplemental Data



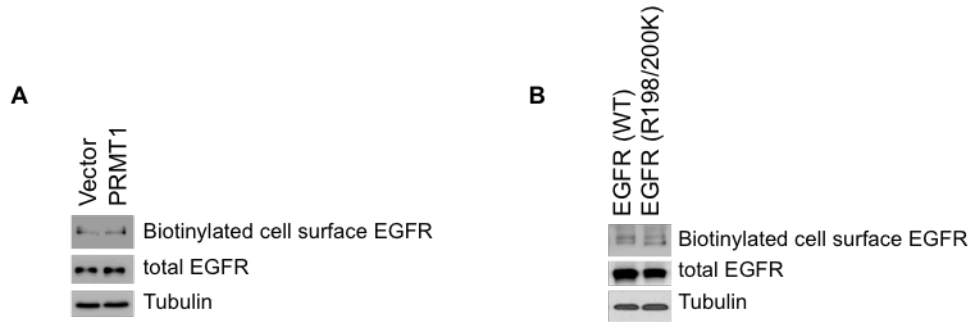
Supplemental Figure 1. Methylation on EGFR extracellular domain. Mass spectrum analysis showing methylation of immunopurified endogenous EGFR at (**A**) R29, (**B**) R74, (**C**) R198 and R200, (**D)** R285, and (**E**) R497. Data shown are representative of 3 independent experiments.


A
PRMT1-EGFR interaction (red)
PDI (green)
Merge (co-localization : yellow and orange)
B
PRMT1-EGFR interaction (red)
SDF4 (green)
Merge (co-localization : yellow and orange)
C
HA (red)
PDI (green)
Merge
PRMT1-HA (wild type)
PRMT1-HA (ΔN)
D
HA (red)
SDF4 (green)
Merge
PRMT1-HA (wild type)
PRMT1-HA (ΔN)
E
PRMT1-HA (WT): - + -
PRMT1-HA (ΔN): - - +
Methyl-EGFR
EGFR
HA
Tubulin
F
SAM (red)
PDI (green)
Merge
G
SAM (red)
SDF4 (green)
Merge

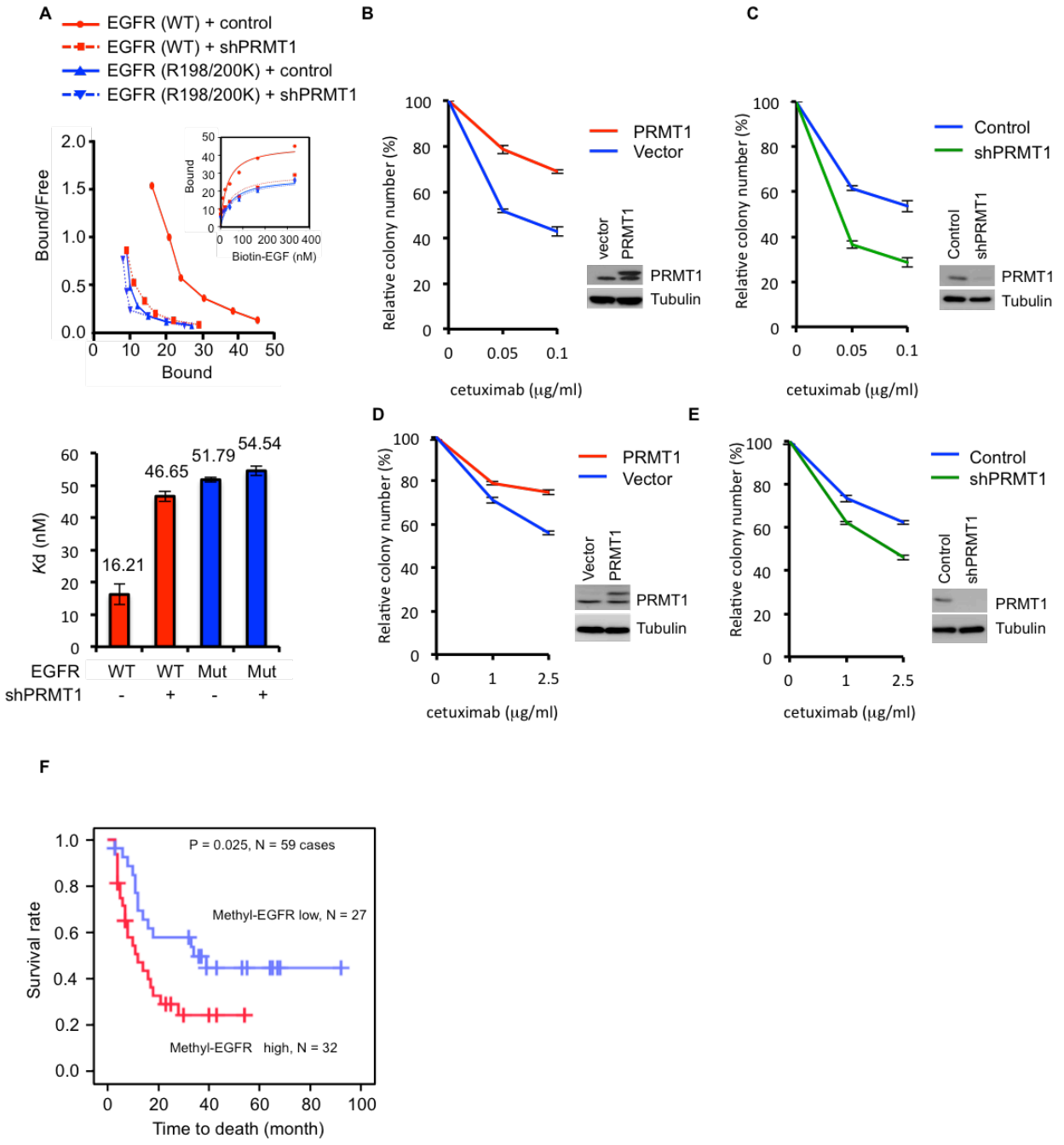
Supplemental Figure 2. PRMT1 interacts with EGFR in ER/Golgi compartment. (A) Immunofluorescence staining of ER lumen protein, PDI, and Duolink assay of endogenous EGFR and PRMT1 in SKCO1 cells. Red spots represent the interaction between PRMT1 and EGFR. (B) Immunofluorescence staining of Golgi lumen protein, SDF4, and Duolink assay of endogenous EGFR and PRMT1 in SKCO1 cells. (C) Immunofluorescence staining of PDI, exogenously expressed HA-tagged PRMT1 and N-terminal deletion (ΔN) mutant HA-tagged PRMT1 in Hela cell. (D) Immunofluorescence staining of SDF4, exogenously expressed HA-tagged PRMT1 and N-terminal deletion (ΔN) mutant HA-tagged PRMT1 in HeLa cells. (E) Immunoblot assessing methyl-EGFR, endogenous EGFR and exogenously expressed PRMT1 levels in Hela cells. (F and G) Immunofluorescence staining of PDI (F), SDF4 (G), and endogenous SAM in HeLa cells. Data shown are representative of 3 independent experiments. For all panels: scale bar, 10 μm. For all insets: 9x magnified images of boxed area; scale bar, 5 μm.



Supplemental Figure 3. PRMT1-mediated upregulation of EGFR signaling can be stimulated by TGFα and requires the enzymatic activity of PRMT1. (A) Immunoblots comparing pEGFR and pERK levels upon TGFα stimulation for 20 min in GEO cells expressing PRMT1 or control vector. **(B)** Immunoblots comparing pEGFR and pERK levels upon EGF stimulation for 20 min in SKCO1 cells expressing control vector, wild type or catalytically inactive mutant PRMT1. Blots shown are representative of 4 independent experiments.

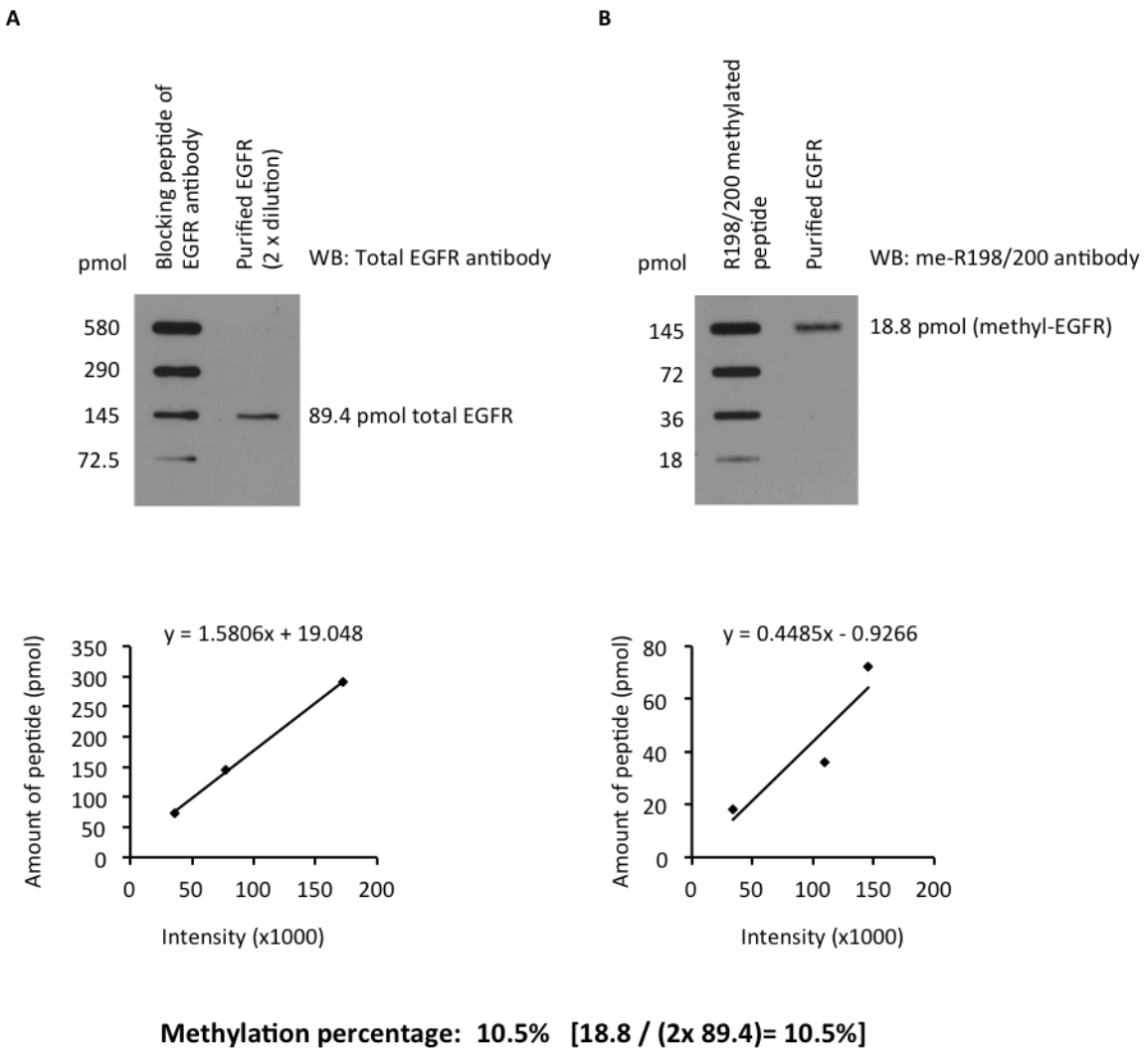


Supplemental Figure 4. EGFR methylation does not affect its cell surface expression level. (**A** and **B**) Biotinylated cell surface EGFR from (**A**) SKCO1 and (**B)** GEO cells were captured on streptoavidin-agarose beads and detected by immunoblot. Blots shown are representative of 3 independent experiments.



Supplemental Figure 5. EGFR R198/200 methylation regulates EGF binding to EGFR and increases cetuximab resistance. (**A**) Top: Scatchard plot and binding curves (inset) which measured EGFR-EGF binding affinity of SW48 cells expressing WT or R198/200K mutant EGFR with or without knocking down of PRMT1. Bottom: Bar graph of *K*d values, Red: WT

EGFR, Blue: R198/200K mutant EGFR. (**B** and **C**) Clonogenic assay of (**B**) GEO cells expressing control vector, exogenous PRMT1 or (**C**) PRMT1 shRNA under cetuximab treatment (N = 3). (**D** and **E**) Clonogenic assay of **(D)** SW48 cells expressing control vector, exogenous PRMT1 or **(E)** PRMT1 shRNA under cetuximab treatment (N= 3). All data are expressed as mean ±SD. Expression levels of PRMT1 shown by immunoblot. Data shown are representative of 4 independent experiments. (**F**) Kaplan-Meier plots of overall survival of 59 head and neck cancer cases after cetuximab treatment with low or high methyl-EGFR level detected by me-R198/200 Ab.



Methylation percentage: 10.5% [18.8 / (2x 89.4)= 10.5%]

Supplemental Figure 6. Stoichiometry of EGFR methylation. (A) Dot blot of blocking peptide of EGFR antibody as well as immuno-purified endogenous EGFR from SKCO1 cells. Signals were detected by EGFR antibody. **(B)** Dot blot of R198/200 methylated peptide as well as immuno-purified endogenous EGFR from SKCO1 cell. Signals were detected by me-R198/200 antibody. Data shown are representative of 3 independent experiments.

Supplemental Table 1. Relationship between expression of PRMT1 and methyl-EGFR in head and neck cancer specimens from 59 cetuximab-treated patients.

		Methyl-EGFR			
		Low	High	Total	*P* value
PRMT1	Low	19 (32.2%)	3 (5.1%)	22 (37.3%)	0.0001*
	High	8 (13.5%)	29 (49.2%)	37 (62.7%)	
	Total	27 (45.8%)	32 (54.2%)	59 (100%)	

*Correlation between PRMT1 and methyl-EGFR was analyzed using the spearman's rank correlation test (*P* = 0.0001). A *P* value of less than 0.05 was set as the criterion for statistical significance.