

Supplementary Materials for

Activation of ERBB2 signaling causes resistance to the EGFR-directed therapeutic antibody cetuximab

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References

Oligonucleotide sequences

ERBB2 Site directed mutagenesis oligos:

Forward primer: 5' -
GAAAATTCCAGTGGCCATCATGGTGTGAGGGAAAACACATC-3'

Reverse primer: 5'-
GATGTGTTTTCCCTCAACACCATGATGGCCACTGGAATTTTC-3'

HRG quantitative PCR primers:

HRG forward primer: 5'-CGTGGAATCAAACGAGATCATCAC-3'

HRG reverse primer: 5'-GGTTATGGTCAGCACTCTCTTCTGG-3'

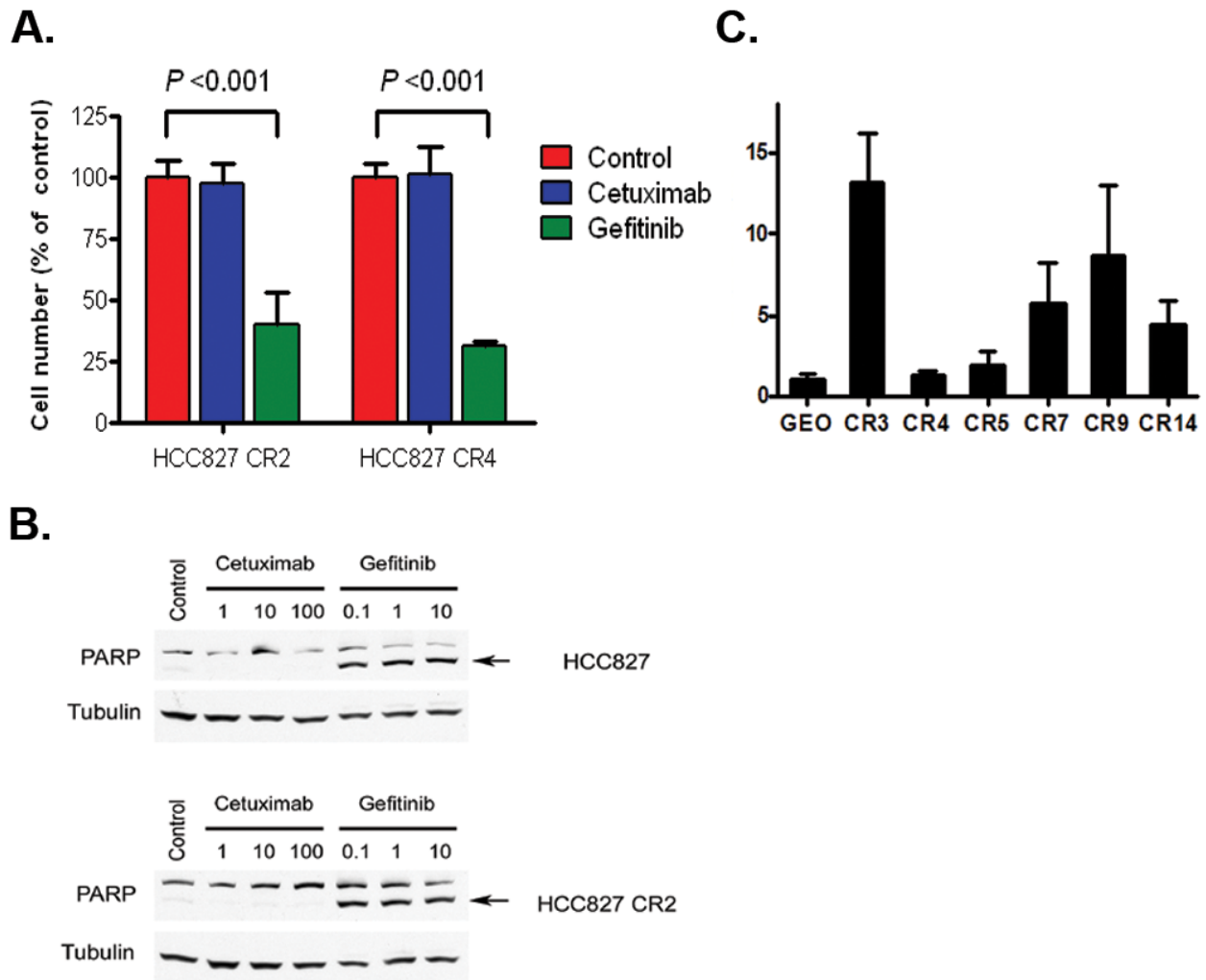
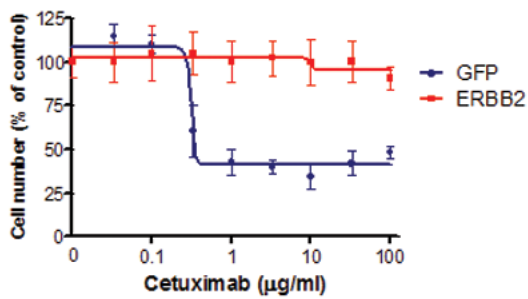
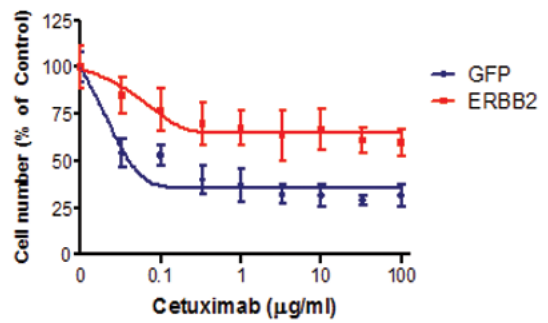
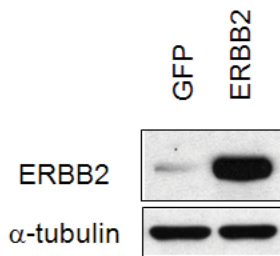


Figure S1. Cetuximab resistant HCC827 and GEO cells. **A.** HCC827 CR2 and CR4 cells were treated with cetuximab (100 $\mu\text{g}/\text{ml}$) or gefitinib (1 μM) and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B.** Control, gefitinib (μM) or cetuximab ($\mu\text{g}/\text{ml}$) treated parental or CR2 cells were treated for 48 hours and cell extracts were immunoblotted to detect indicated proteins. Arrow; cleaved PARP **C.** Quantitative PCR for *ERBB2* from GEO and cetuximab resistant subclones



HCC827



H1648

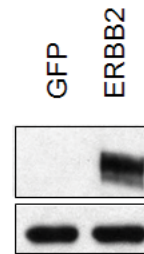


Figure S2. ERBB2 causes resistance to cetuximab in cetuximab sensitive cells. ERBB2 or GFP were introduced into HCC827 or H1648 by retroviral infection. The resulting cells were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. ERBB2 expression is confirmed by immunoblotting.

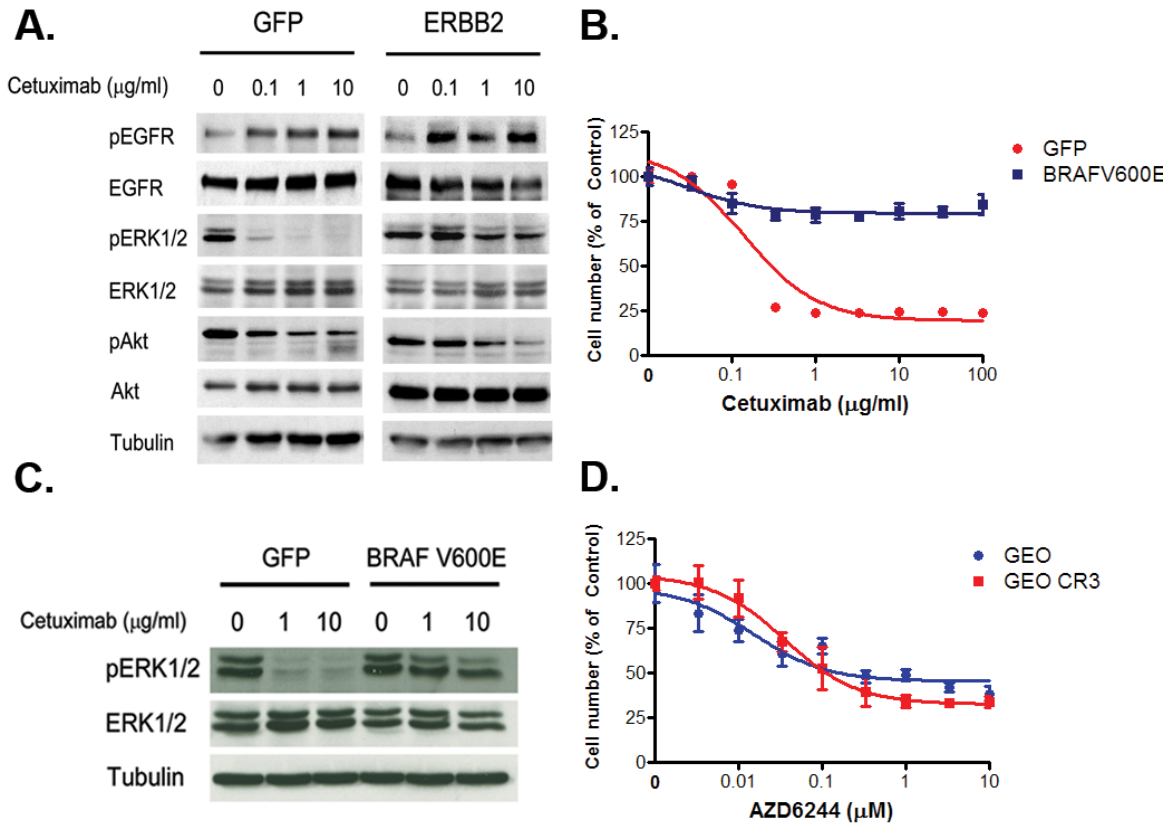


Figure S3. ERBB2 maintains ERK 1/2 signaling in the presence of cetuximab. **A.** HCC827 GFP and HCC827 ERBB2 cells were treated with indicated concentrations of cetuximab for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **B.** HCC827 cells expressing GFP or BRAFV600E were treated with cetuximab at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **C.** Cells from **B.** were treated with indicated concentrations of cetuximab for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **D.** GEO and GEO CR3 cells were treated with increasing concentrations of AZD6244 and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls

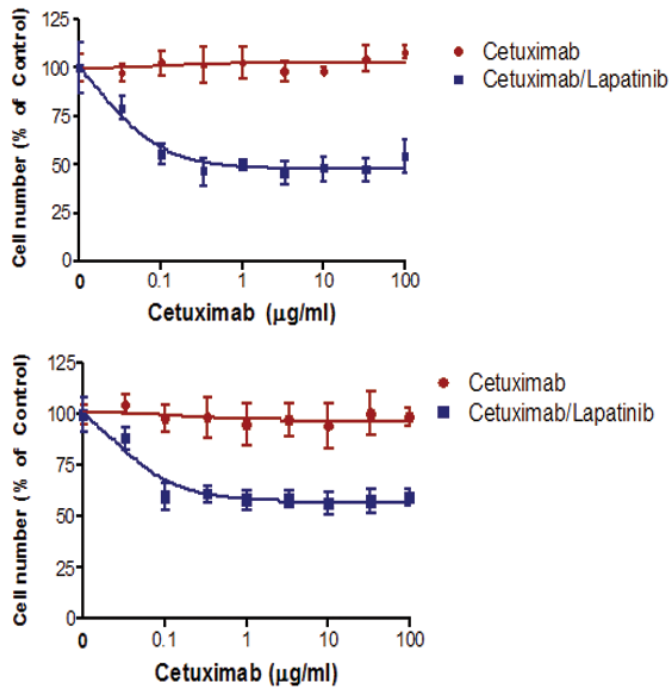
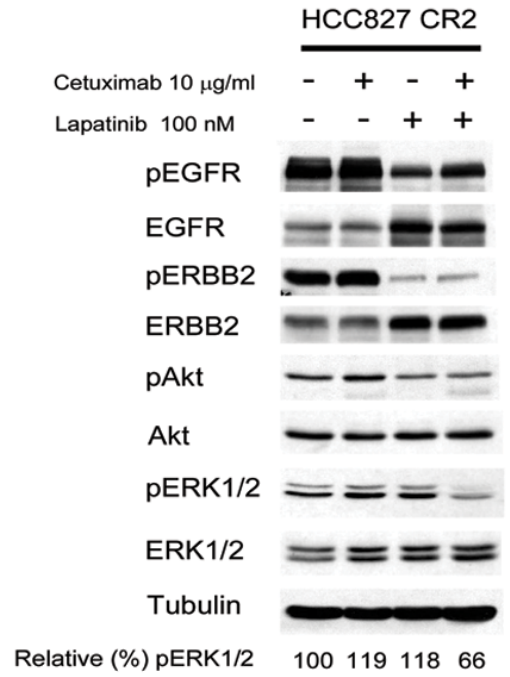
A.**B.**

Figure S4. Lapatinib restores sensitivity to cetuximab. **A.** HCC827 CR2 and CR4 cells were treated with cetuximab alone, or in combination with 100 nM lapatinib, at the indicated concentrations, and viable cells were measured after 72 hours of treatment and plotted relative to untreated controls. **B.** HCC827 CR2 cells were treated with cetuximab (10 µg/ml) alone, lapatinib alone (100 nM) or with both drugs for 6 hours. Cell extracts were immunoblotted to detect indicated proteins.

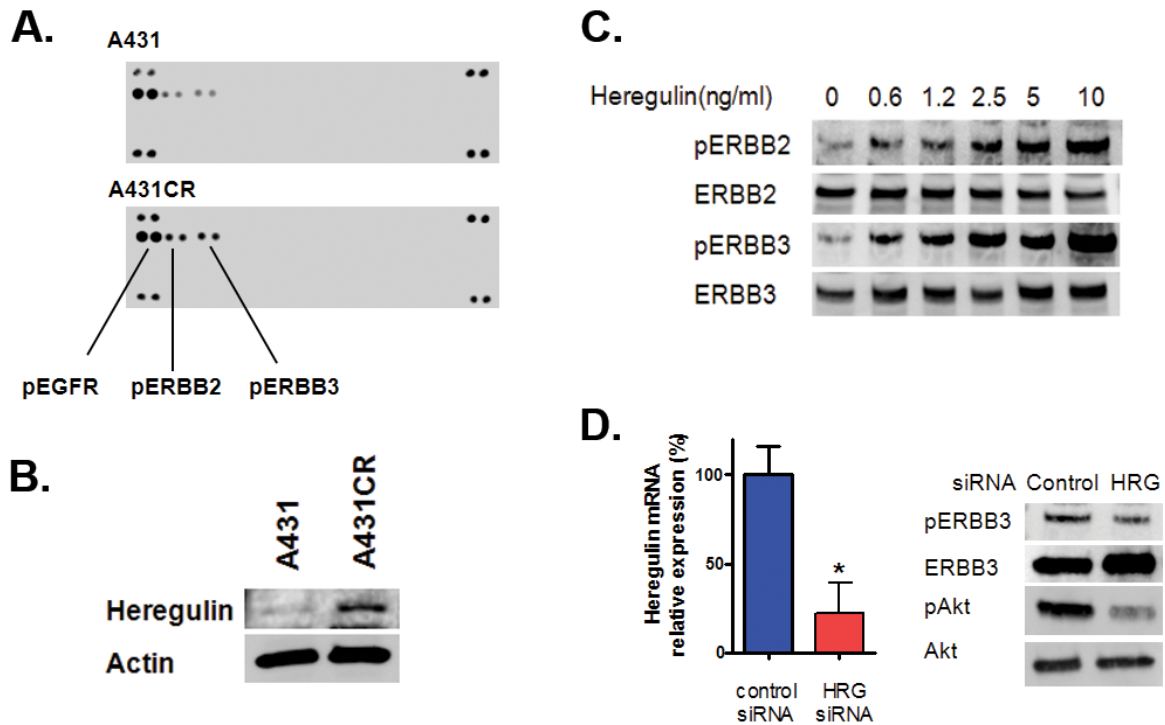


Figure S5. Heregulin mediates resistance to cetuximab in A431 cells. **A.** Parental A431 and resistant A431CR cells were lysed, and the whole cell lysates were hybridized to a phospho-RTK array. In the array, each RTK was spotted in duplicate. Hybridization signals at the corners served as controls. **B.** A431CR cells express greater amounts of heregulin. Cell extracts were immunoblotted to detect indicated proteins. **C.** A431 cells were exposed to indicated concentrations of heregulin for 6 hours. Cell extracts were immunoblotted to detect indicated proteins. **D.** Control or heregulin (HRG) siRNAs were transfected into A431CR cells. Heregulin mRNA was measured by quantitative PCR as described in Materials and Methods. *, $p=0.0044$ (unpaired t test). Cell extracts isolated from the cells were immunoblotted to detect indicated proteins.

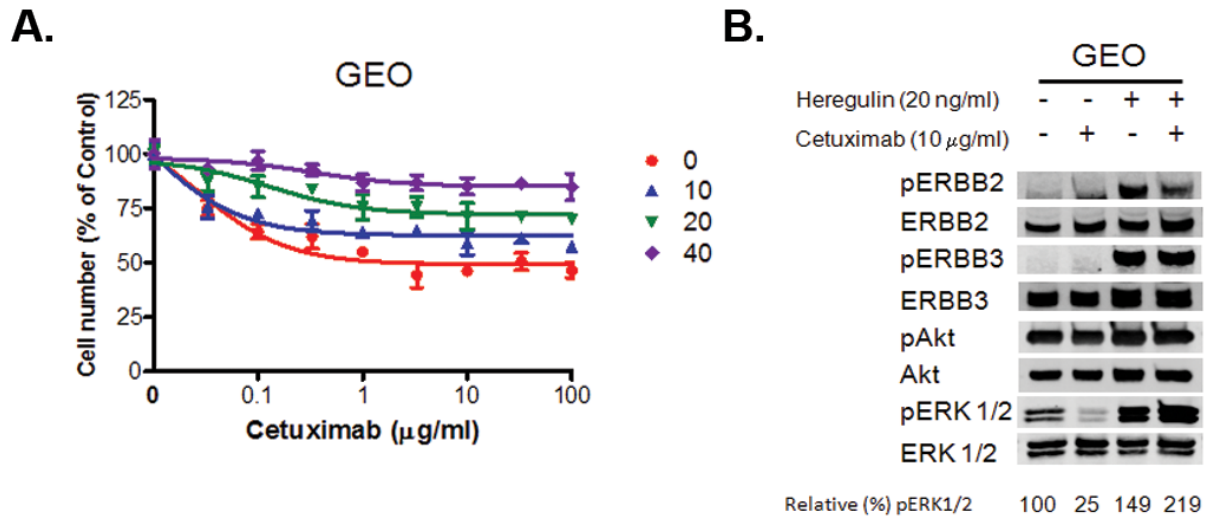


Figure S6. Heregulin mediates resistance to cetuximab in the GEO cells. **A.** GEO cells were treated with cetuximab at the indicated concentrations in the presence of heregulin at the indicated concentrations (ng/ml). The percentage of viable cells is shown relative to untreated controls. **B.** GEO cells were treated with cetuximab (10 μg/ml) alone, heregulin alone (20 ng/ml) or the combination. Cells were lysed, and the indicated proteins were detected by immunoblotting.

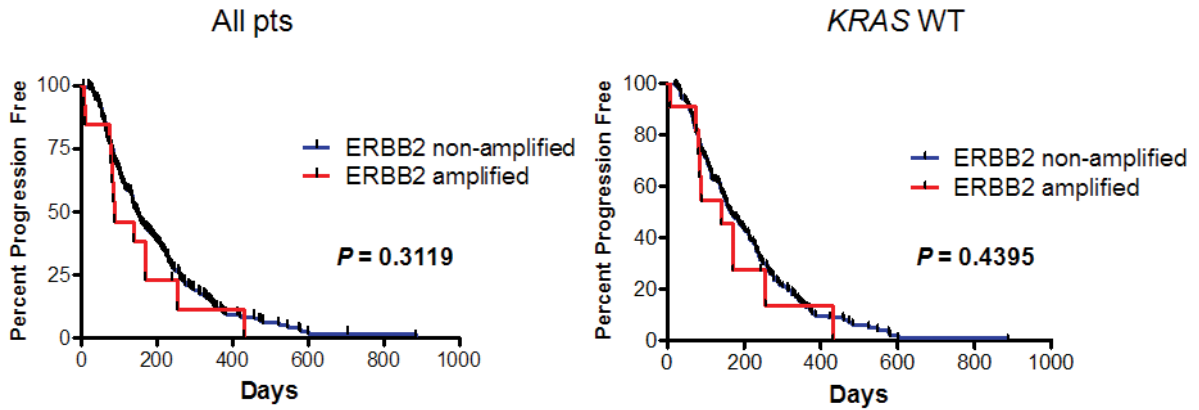


Figure S7. Progression free survival for all CRC patients treated with cetuximab based therapy. Progression free survival for all (left) CRC patients with (n = 13) and without *ERBB2* amplification (n = 220) treated with cetuximab based therapy. (Right) Data for *KRAS* wild type only patients (*ERBB2* amplified; n = 11; *ERBB2* non-amplified; n = 171). Comparison based on log-rank test.

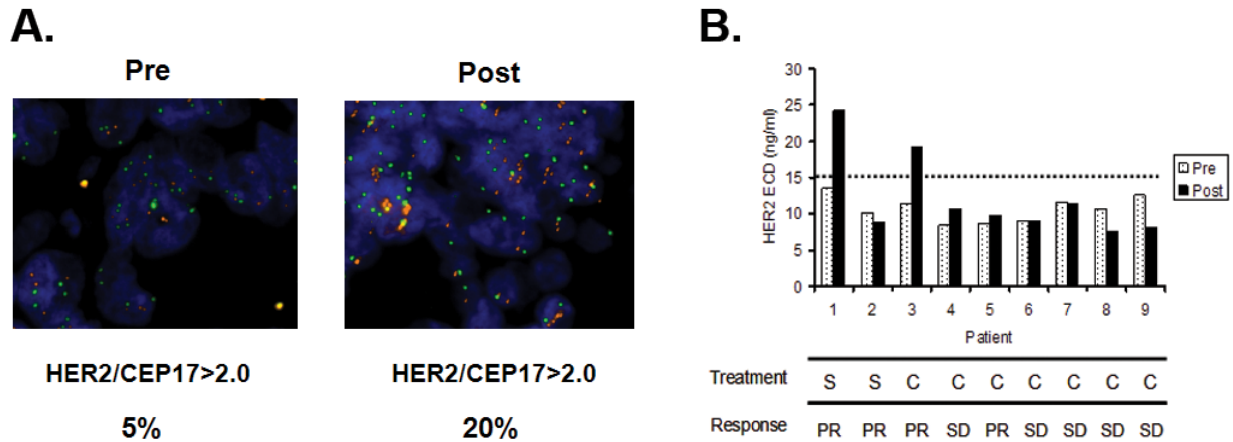


Figure S8. Increased *ERBB2* copy number is associated with acquired cetuximab resistance. **A.** Increased *ERBB2* copy number in cetuximab resistant tumor specimen. *ERBB2* FISH from a baseline tumor specimen (left) and following acquired cetuximab resistance (right). *ERBB2* (red) and CEP 17 (green). **B.** Serum levels of the *ERBB2* extracellular domain (ECD) from colorectal cancer patients before and after cetuximab-based therapy. Dotted line, 15 ng/ml (cutoff for abnormal). S, single agent cetuximab; C, combination with chemotherapy; PR, partial clinical response; SD, stable disease.

Characteristic	No. of patients
<i>Gender</i>	
Male	145
Female	88
<i>Prior treatment regimens</i>	
None	16
1 prior regimen	81
2 prior regimens	91
3 prior regimens	43
> 3 prior regimens	2
<i>EGFR directed therapy</i>	
Cetuximab alone	7
Cetuximab/irinotecan	166
Cetuximab/oxaliplatin	47
Cetuximab/bevacizumab	2
Cetuximab/other chemotherapy	4
Panitumumab alone	3
Panitumumab/irinotecan/oxaliplatin/5FU	4
<i>ERBB2</i> amplified	13
<i>ERBB2</i> non-amplified	220
<i>KRAS</i> mutant	49
<i>KRAS</i> wild type	184

Table S1. Characteristics of colorectal cancer patients used to evaluate impact of

***ERBB2* amplification.** 5FU; 5-Fluorouracil. The cohort consisted of 262 patients; FISH for *ERBB2* amplification was possible in 233/262 (89%) of patients.

Patient	Prior Therapies	Treatment	Best response	Duration (days)
1	2	Cetuximab	PR	147
2	2	Cetuximab	PR	182
3	3	Cetux/irinotecan	PR	416
4	3	Cetux/irinotecan	SD	161
5	2	Cetux/irinotecan	PR	182
6	2	Cetux/irinotecan	SD	70
7	4	Cetux/irinotecan	SD	201
8	3	Cetux/irinotecan	SD	154
9	2	Cetux/irinotecan	SD	83

Table S2. Clinical and treatment information on colorectal cancer patients used for plasma ERBB2 extracellular domain measurements.

Characteristic	No. of patients
<i>Gender</i>	
Male	45
Female	25
<i>Prior treatment regimens</i>	
None	0
1 prior regimen	6
2 prior regimens	54
3 prior regimens	6
> 3 prior regimens	4
<i>EGFR directed therapy</i>	
Cetuximab alone	21
Cetuximab/irinotecan	49
<i>Response to Cetuximab Therapy</i>	
Partial Response	16
Stable disease or progressive disease	49
Not evaluable for response	5
<i>KRAS wild type</i>	
<i>KRAS</i> wild type	42
<i>KRAS</i> mutant	11
<i>KRAS</i> unknown	17

Table S3. Characteristics of colorectal cancer patients used for plasma and tumor based studies of heregulin.