

Supplementary Information

Cytokine and epigenetic regulation of CEACAM6 mediates EGFR-driven signaling and drug response in lung adenocarcinoma

Ming-Yi Zheng^{1,5}, Yen-Ting Lin^{1,5}, Yen-Shou Kuo^{2,5}, Yen-Ju Lin¹, Ming-Han Kuo¹, Tsai-Wang Huang², Yi-Shing Shieh³, Yen-Lin Huang⁴, Yu-Ting Chou^{1*}

¹Institute of Biotechnology, National Tsing Hua University, Hsinchu, Taiwan.

²Division of Thoracic Surgery, Department of Surgery, Tri-Service General Hospital, National Defense Medical Center, Taipei, Taiwan.

³Department of Oral Diagnosis & Pathology, Tri-Service General Hospital, Taipei, Taiwan.

⁴Department of Medicine, National Tsing Hua University, Hsinchu, and Department of Anatomic Pathology, Chang Gung Memorial Hospital Linkou, Taoyuan, Taiwan.

⁵Co-first authors

Contact: *Address correspondence to: Dr. Yu-Ting Chou, National Tsing Hua University, 101, Section 2, Kuang-Fu Road, Hsinchu, 300 Taiwan. Phone: 886-3-574-2471. Fax: 886-3-571-5934. E-mail: ytchou@life.nthu.edu.tw

Supplementary Table 1. STR analysis of HCC827 and HCC827GR cells.

Results : Matched to HCC-827 Cell line.

HCC827	Locus names												
	D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO				
	12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11				
Database													
ATCC DSMZ JCRB RIKEN ECACC ExPASy													
	EV	Cell No.	Cell name	Locus names									
				D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO	
		Query (Your Cell)		12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11	
		1.00(36/36)	566	HCC-827	12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11
		1.00(36/36)	CRL-2868	HCC827	12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11

Searching the genotypes of the sample in the ATCC / DSMZ / JCRB / RIKEN/ ECACC / ExpASY STR database, the test sample result matched to HCC-827 Cell line.

Results : Matched to HCC-827 Cell line.

HCC827GR	Locus names												
	D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO				
	12,12	9,9	12,12	12,12	18,18	6,6	X,X	8,8	11,11				
Database													
ATCC DSMZ JCRB RIKEN ECACC ExPASy													
	EV	Cell No.	Cell name	Locus names									
				D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO	
		Query (Your Cell)		12,12	9,9	12,12	12,12	18,18	6,6	X,X	8,8	11,11	
		0.94(34/36)	566	HCC-827	12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11
		0.94(34/36)	CRL-2868	HCC827	12,12	9,9	11,12	12,12	18,18	6,6	X,X	8,8	11,11

Searching the genotypes of the sample in the ATCC / DSMZ / JCRB / RIKEN/ ECACC / ExpASY STR database, the test sample result matched to HCC-827 Cell line.

Supplementary Table 2. TaqMan qPCR primers.

Primer	Sequence (5'-3')	Probe
CEACAM5_F	ACCACAGTCACGACGATCAC	UPL#9
CEACAM5_R	CTCCACGGGGTTGGAGTT	
CEACAM6_F	GCATCACGATTGGAGTGCT	UPL#21
CEACAM6_R	TTCAGGGTCTGGTCCAATCT	
CDH1_F	CAGGCTCAAGCTATCCTTGC	UPL#33
CDH1_R	AGTCATGCGTAGTGGTGCAT	
VIM_F	TACAGGAAGCTGCTGGAAGG	UPL#15
VIM_R	CCAGAGGGAGTGAATCCAGA	
EPCAM_F	CCATGTGCTGGTGTGTGAA	UPL#3
EPCAM_R	TGTGTTTTAGTTCAATGATGATCC	
SNAI2_F	TGGTTGCTTCAAGGACACAT	UPL#7
SNAI2_R	GCAAATGCTCTGTTGCAGTG	
AXL_F	GTGGCCCTGGTGGTATGTAC	UPL#27
AXL_R	GGTGGACAAGGAAGAGAGCC	
SMAD4_F	TGGGGAGTCCTGGTAGAGG	UPL#55
SMAD4_R	CAACAGCAGCTGGTTTCATC	
TGFBR1_F	GCAAATAAATTACATTTTTCCCAAG	UPL#59
TGFBR1_R	CAGCAACTACATATGGCATGACT	
TGFBR2_F	ATGAGGAAAGAGGCTCCATGT	UPL#76
TGFBR2_R	TGCAAAAGCAAGTGCAATGT	
TGFB1_F	GGTCTCCATCCCTGACGTT	UPL#16
TGFB1_R	GGGCAAAGGAATAGTGCAGA	
TGFB2_F	AGGAAGGGGTGAAGTGCTAGT	UPL#44
TGFB2_R	CAACGATCAAATTGTACTGCTGA	
TGFB3_F	CTGGCCCTTTACAACAGCAC	UPL#25
TGFB3_R	TCCGACTCGGTGTTTTCT	

18S_F	TGGCTCATTAAATCAGTTATG	CGCTCGCT CCTCTCCTA CTTG
18S_R	CGGCATGTATTAGCTCTA	

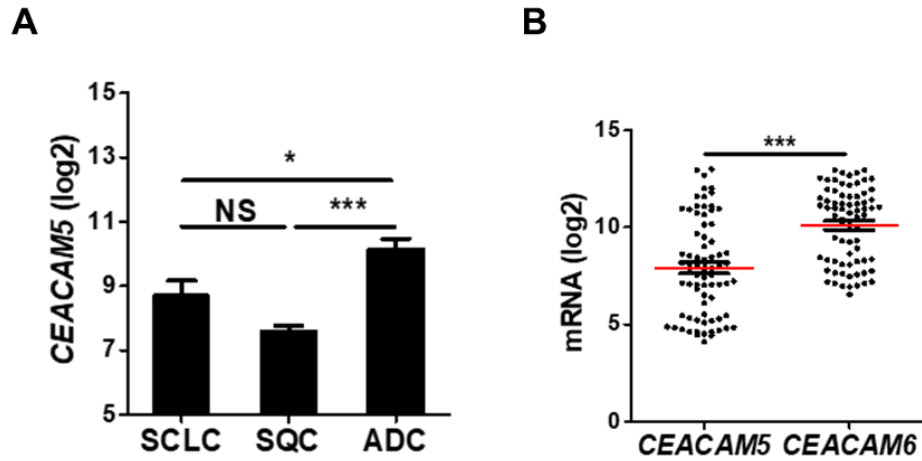
Supplementary Table 3. ChIP-qPCR primers.

Primer	Sequence (5'-3')	Probe
Primer1_F	TTGTTGTAATTCTGTCTCTTGATGTG	UPL#76
Primer1_R	CCGCCTGAGAGACTCCACT	
Primer2_F	GGGGAGCACTGAGCTACAAA	UPL#60
Primer2_R	CATATAAAGGTCCCCAGCACA	

Supplementary Table 4. Gene expression data from public cohorts.

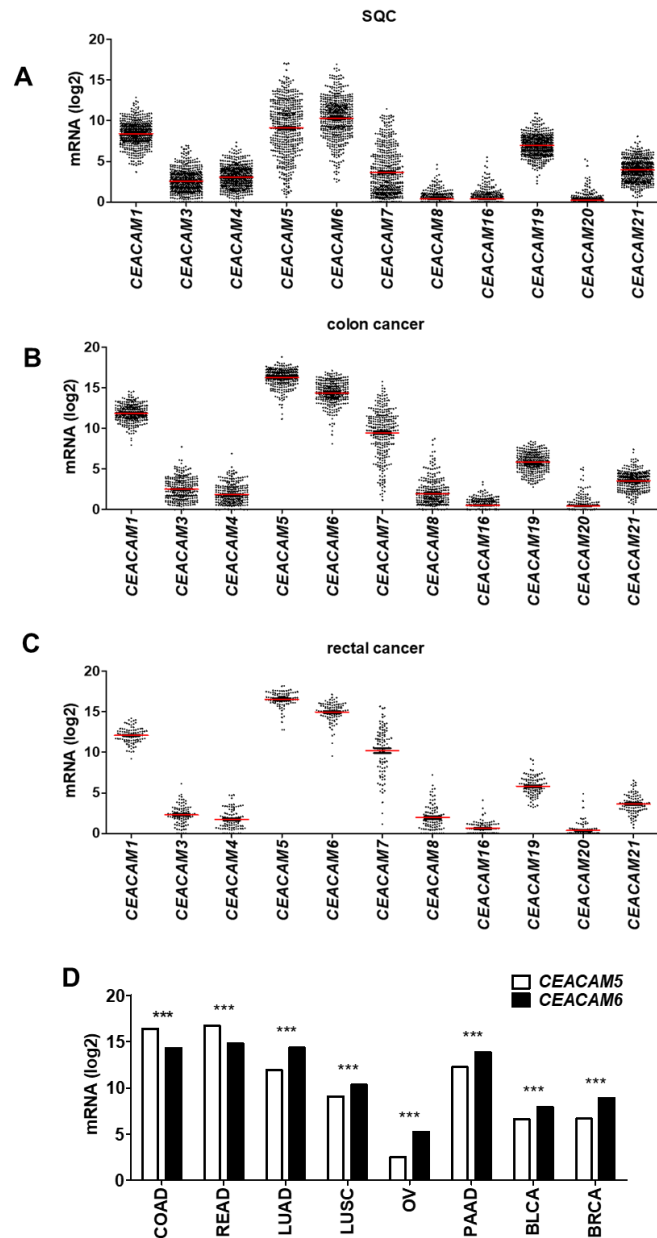
Cohort	Sources
GSE29016	GEO (1)
GSE31552	GEO (2)
GSE2514	GEO (3)
GSE75037	GEO (4)
TCGA	TCGA (5)
TCPA	TCPA (6)
CCLE	CCLE (7)
GSE31210	GEO (8)
GSE38310	GEO (9)
GSE38302	GEO (10)
GSE64322	GEO (11)
GSE289000	GEO (12)
Bivona cohort	Nature (13)
SRX1528527	ChIP-Atlas (14)
SRX2636288	ChIP-Atlas (15)

1. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE29016>
2. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31552>
3. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE2514>
4. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=gse75037>
5. <https://portal.gdc.cancer.gov/analysis>
6. <https://tcpaportal.org/tcpa/>
7. <https://portals.broadinstitute.org/ccle>
8. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE31210>
9. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38310>
10. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE38302>
11. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE64322>
12. <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE289000>
13. <https://www.nature.com/articles/ng.2330>
14. <https://chip-atlas.org/view?id=SRX1528527>
15. <https://chip-atlas.org/view?id=SRX2636288>



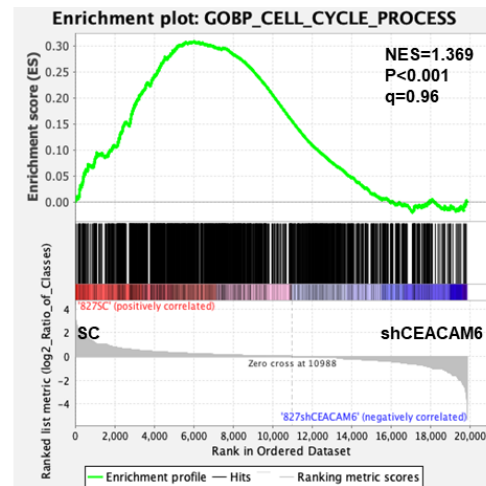
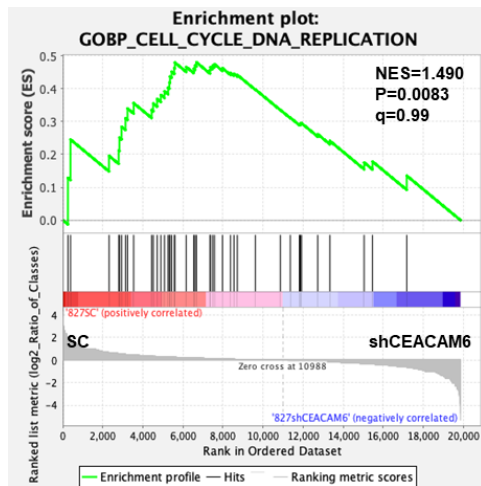
Supplementary Figure 1. Comparison of *CEACAM5* and *CEACAM6* expression in different subtypes of lung cancer.

A. Gene expression profiling analysis assessing *CEACAM5* expression in small cell lung cancer (SCLC, n = 20), lung squamous cell carcinoma (SQC, n = 13), and lung adenocarcinoma (ADC, n = 39) from the GSE29016 database. *, P < 0.05; ***, P < 0.001; NS, non-significant. **B.** Gene expression profiling analysis evaluating *CEACAM5* and *CEACAM6* expression in lung adenocarcinoma (n = 74) from the GSE31552 database. ***, P < 0.001.



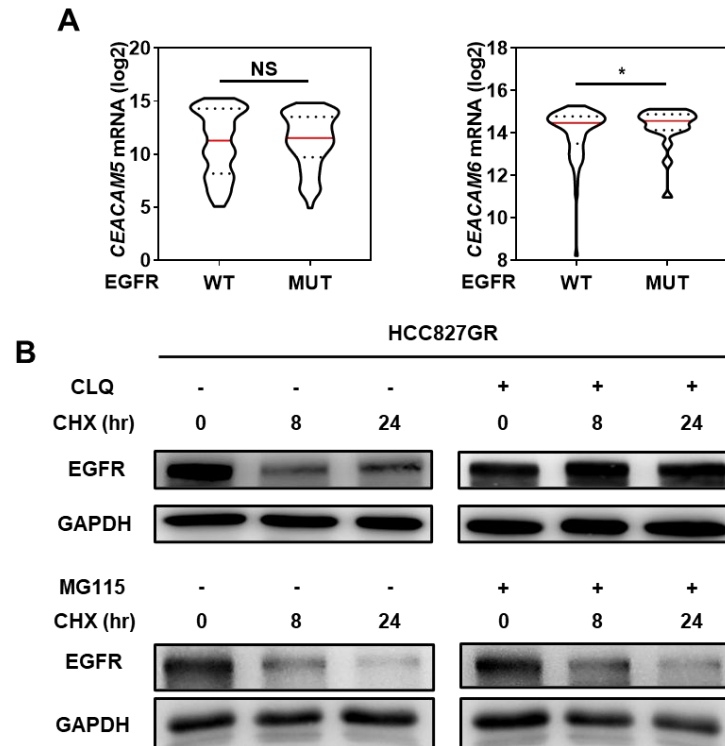
Supplementary Figure 2. CEACAM family member gene expression in cancers.

A-C. RNA-seq analysis assessing CEACAM family member gene expression in (A) lung squamous cell carcinoma (n = 502), (B) colon cancer (n = 288), and (C) rectal cancer (n = 95) from the TCGA database. **D.** RNA-seq analysis assessing *CEACAM5* and *CEACAM6* expression in indicated cancers from the TCGA database. ***, P < 0.001. COAD, Colon adenocarcinoma; READ, Rectum adenocarcinoma; LUAD, Lung adenocarcinoma; LUSC, Lung squamous cell carcinoma; OV, Ovarian serous cystadenocarcinoma; PAAD, Pancreatic adenocarcinoma; BLCA, Bladder urothelial carcinoma; BRCA, Breast invasive carcinoma.



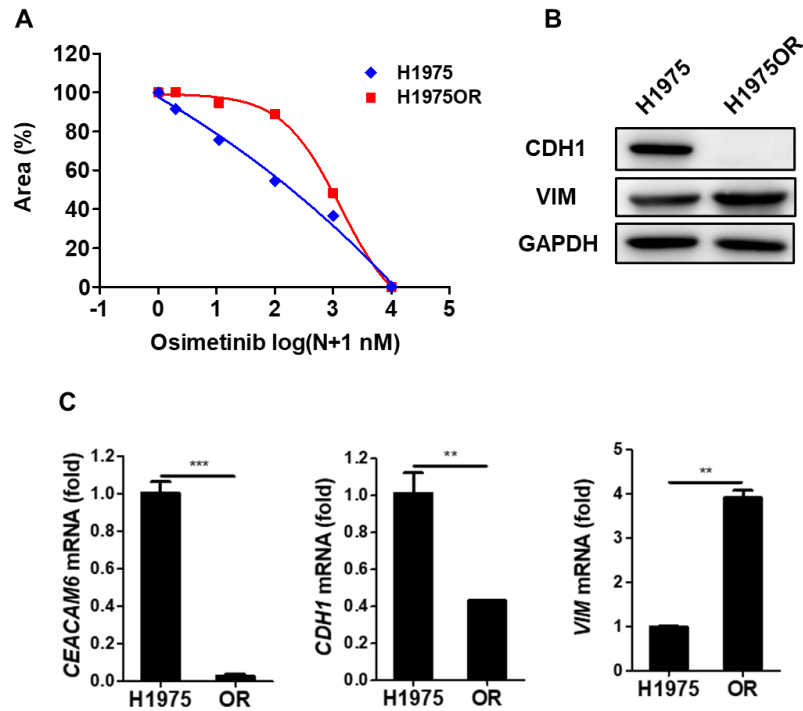
Supplementary Figure 3. Gene Set Enrichment Analysis (GSEA) analysis of the RNA-seq data.

Gene Set Enrichment Analysis (GSEA) analysis of the RNA-seq data revealed downregulation of cell-cycle-related pathways in *CEACAM6*-silenced HCC827 cells.



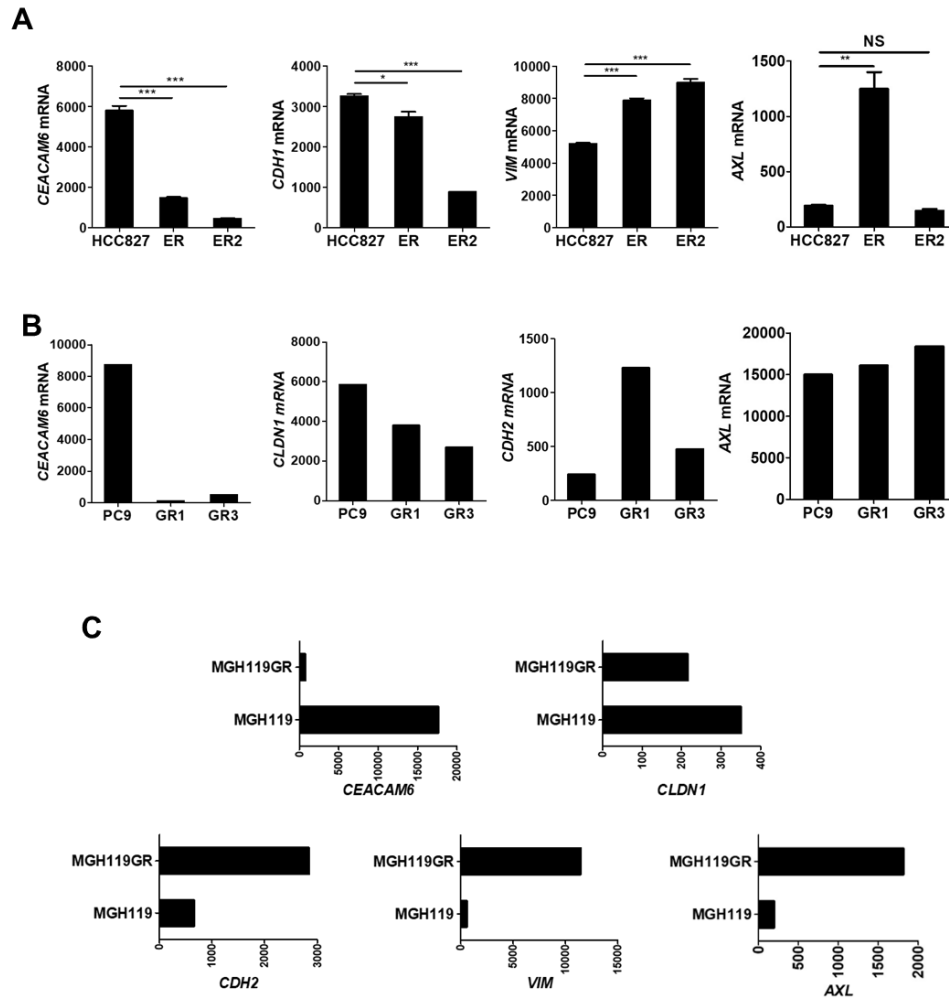
Supplementary Figure 4. CEACAM6 expression and EGFR stability.

A. Gene expression profiling analysis evaluating the correlation of *CEACAM5* (left) and *CEACAM6* (right) expression with *EGFR* status [wild-type (n = 99) versus mutant (n = 127) *EGFR*] in stage I-II lung adenocarcinoma from GSE31210 database. *, P < 0.05. **B.** Immunoblotting analysis assessing EGFR expression in HCC827GR cells pretreated with the autophagy inhibitor chloroquine (50 μ M) or the proteasome inhibitor MG-115 (10 μ M), followed by treatment with cycloheximide (CHX, 50 ng/ml) for the indicated periods.



Supplementary Figure 5. Reverse association of *CEACAM6* expression with EMT.

A. Clonogenic analysis depicting the growth of H1975 and H1975OR cells in the presence of increasing concentrations of Osimertinib for 14 days. H1975OR cells were derived from a pool of H1975 cells that survived incremental concentrations of Osimertinib selection over 6 months. **B.** Immunoblotting analysis assessing CDH1 and VIM expression in H1975 versus H1975OR cells. **C.** qRT-PCR analysis assessing *CEACAM6*, *CDH1*, and *VIM* expression in EGFR-TKI sensitive H1975 and the resistant counterpart H1975OR cells. **, $P < 0.01$.



Supplementary Figure 6. Reverse association of *CEACAM6* expression with EMT in the public domain.

A. Gene expression profiling analysis evaluating *CEACAM6*, *CDH1*, *VIM*, and *AXL* expression in EGFR-TKI sensitive HCC827 versus resistant counterpart HCC827ER1 (ER1) and HCC827ER2 (ER2) cells from the GSE38310 database. *, $P < 0.05$; ***, $P < 0.001$. **B.** Gene expression profiling analysis of *CEACAM6*, the epithelial marker gene *CLDN1*, and the mesenchymal marker gene *CDH2* and *AXL* expression in EGFR-TKI sensitive PC9 versus resistant counterpart PC9GR1 (GR1) and PC9GR3 (GR3) cells from the GSE38302 database. **C.** Gene expression profiling analysis evaluating *CEACAM6*, the epithelial marker gene *CLDN1*, and the mesenchymal marker gene *CDH2*, *VIM*, and *AXL* expression in EGFR-TKI sensitive primary tumor cells (MGH119) versus resistant (MGH119GR) cells from the GSE64322 database.

A			
A549+TGF-β	0h	12h	24h
18s (Ct)	8.97	8.92	8.69
CEACAM5 (Ct)	28.86	29.86	32.49
CEACAM6 (Ct)	24.12	24.60	26.87

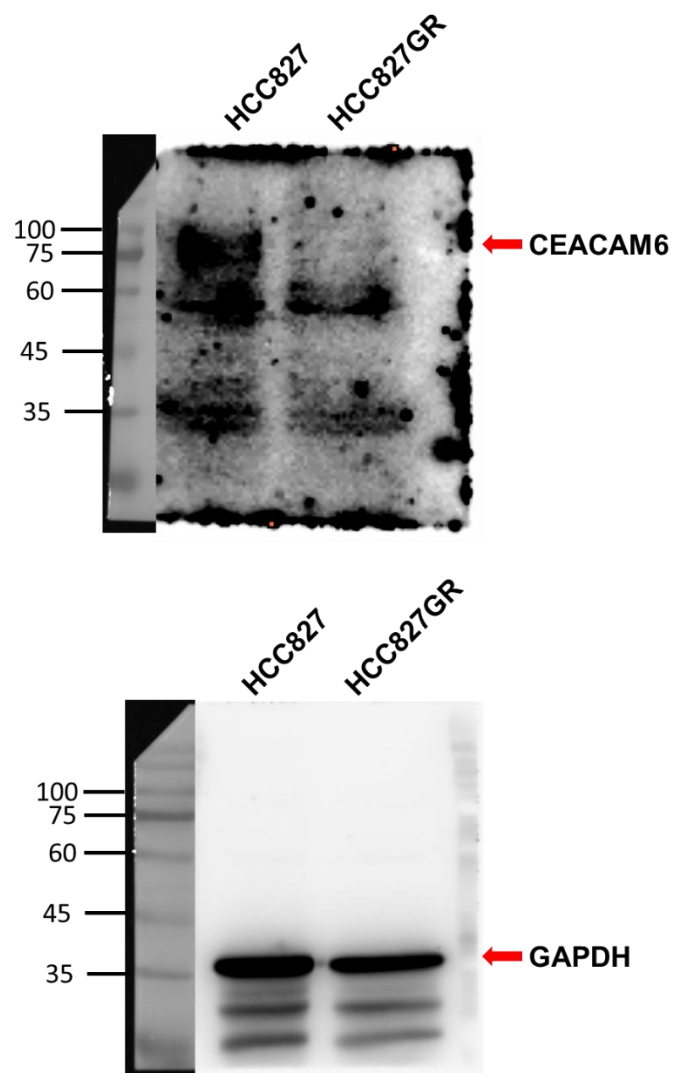
B		
(ng/mL)	A549	A549+TGF-β
CEACAM5	undetectable	undetectable
CEACAM6	1.12	0.14

Supplementary Figure 7. Regulation of *CEACAM6* expression by TGF- β .

A. qRT-PCR analysis evaluating *CEACAM5* and *CEACAM6* expression, represented as the cycle-threshold (Ct) values, in A549 cells treated with TGF- β (1 ng/mL) for 0, 12, and 24 hr. **B.** ELISA analysis assessing CEACAM5 and CEACAM6 protein expression in A549 cells treated with or without TGF- β (1 ng/mL) for 3 weeks.

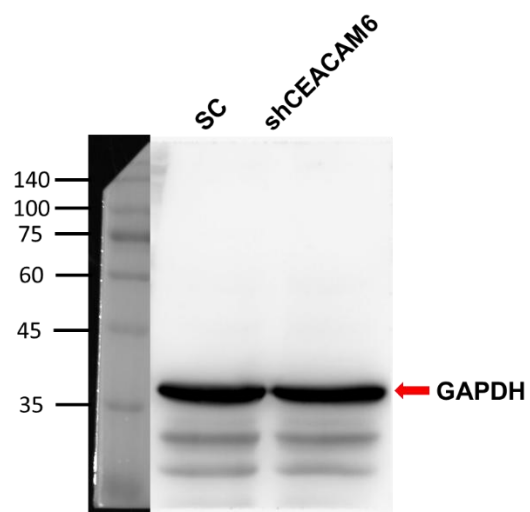
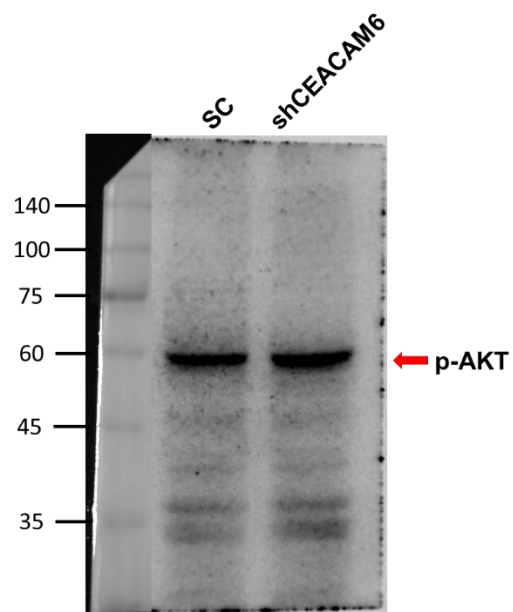
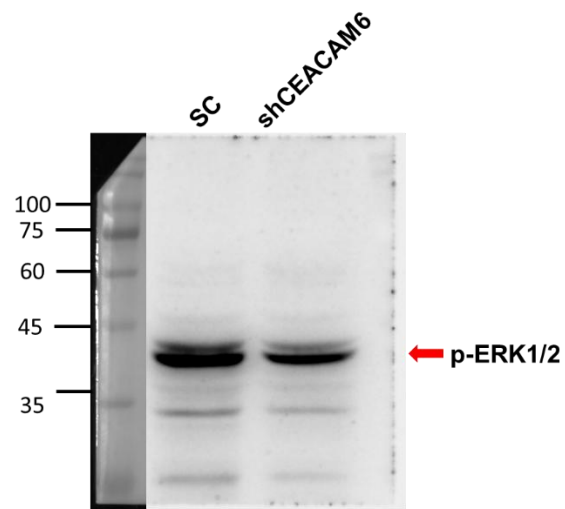
Original Immunoblots

Figure 2A



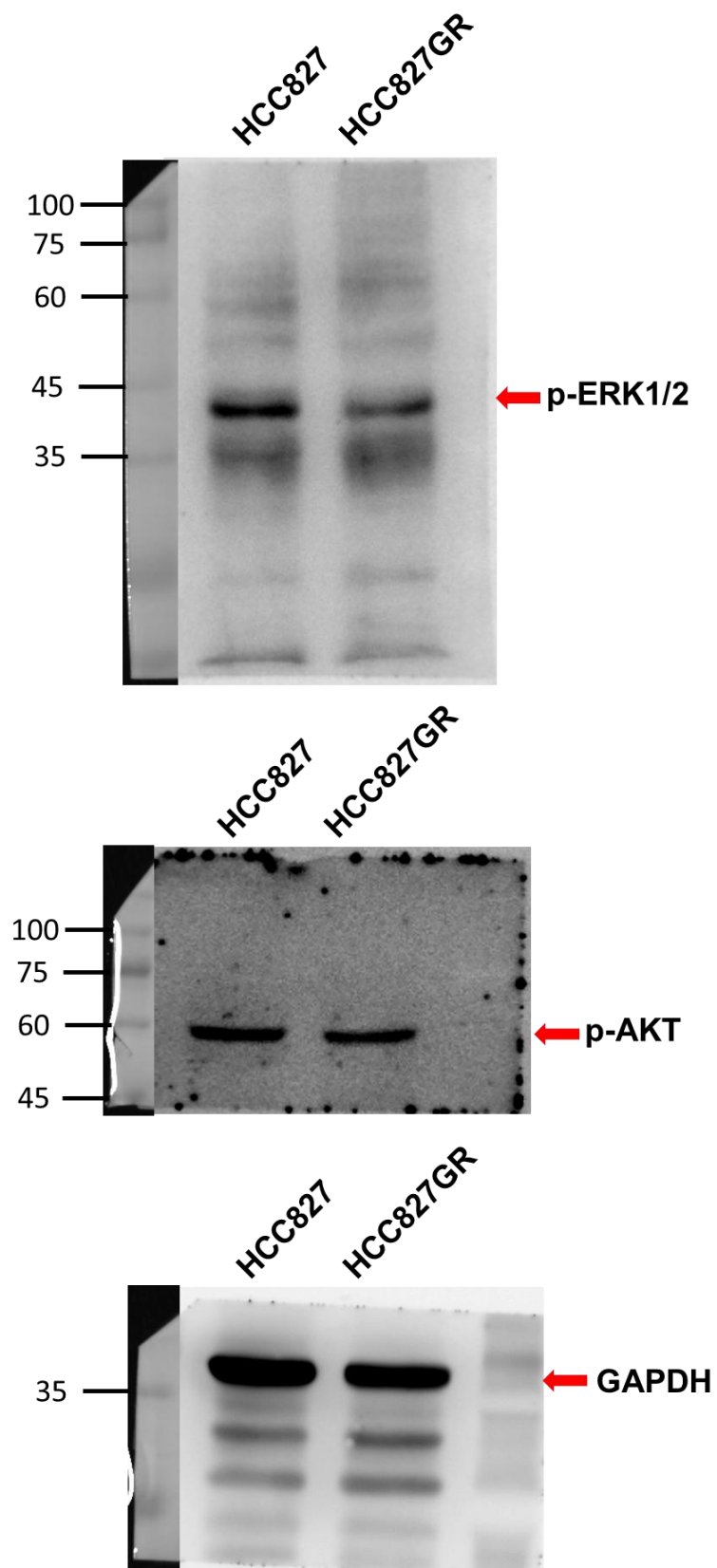
Original Immunoblots

Figure 2D



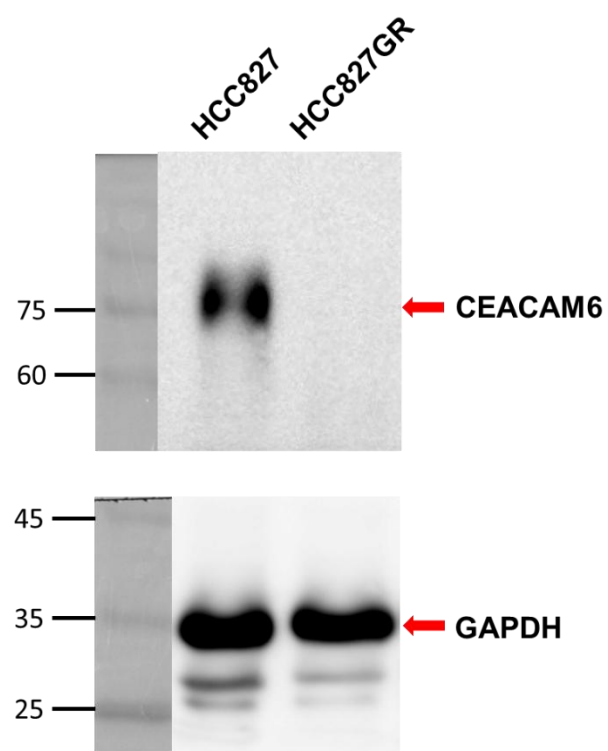
Original Immunoblots

Figure 3B



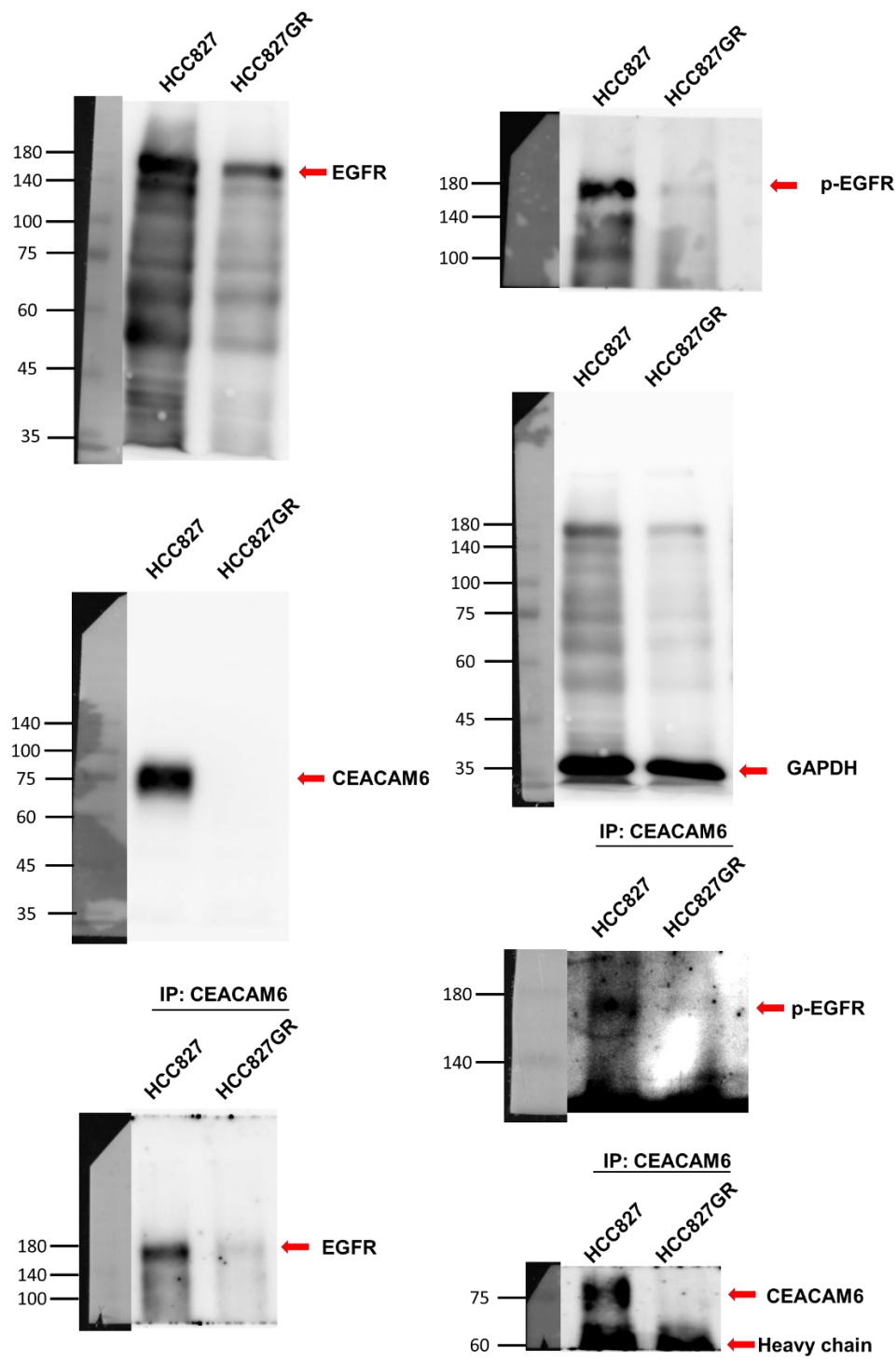
Original Immunoblots

Figure 3E



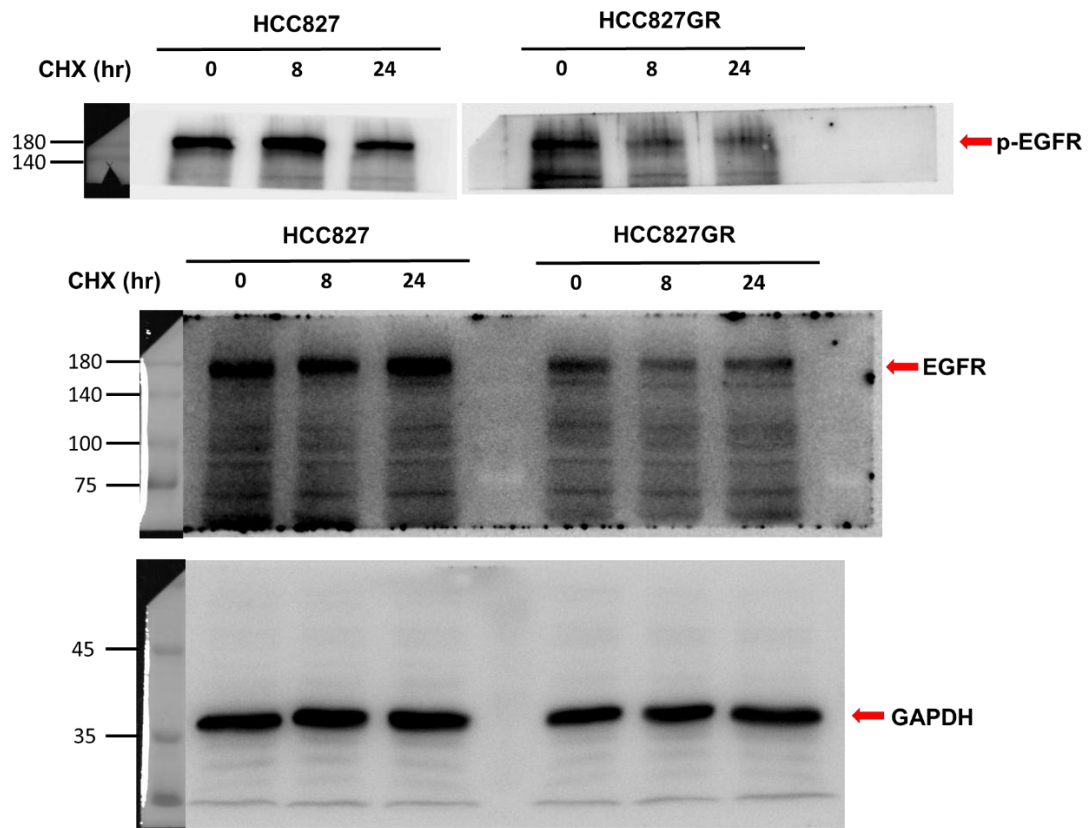
Original Immunoblots

Figure 4D



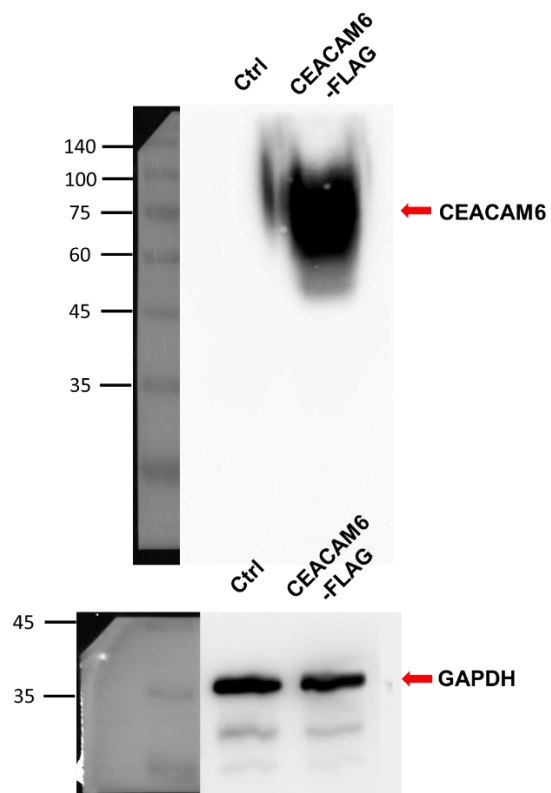
Original Immunoblots

Figure 4E

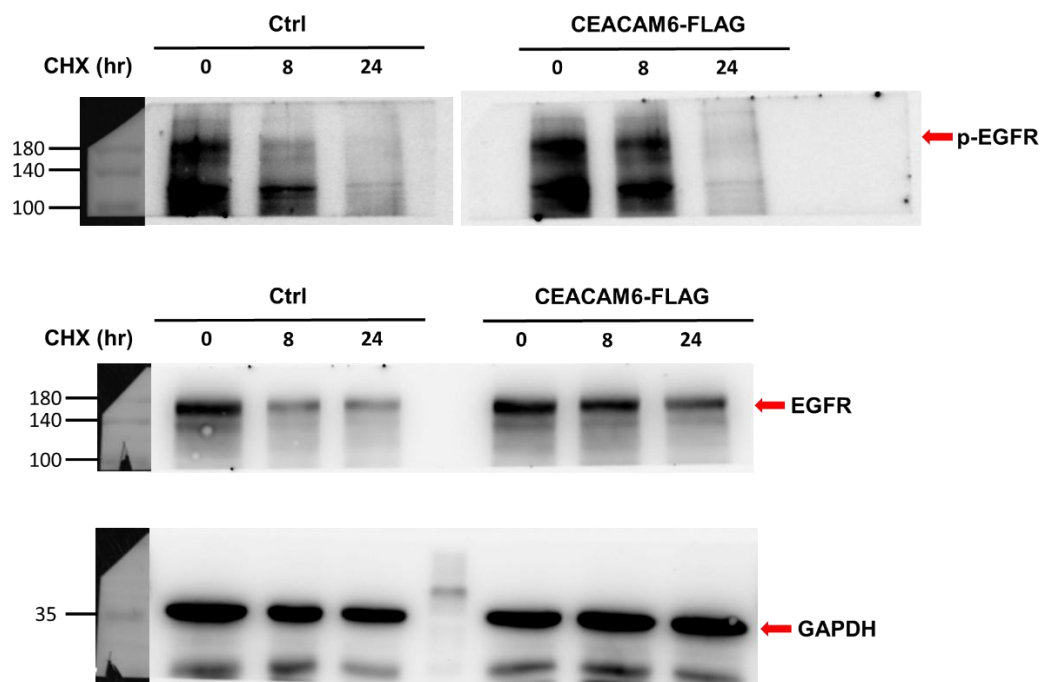


Original Immunoblots

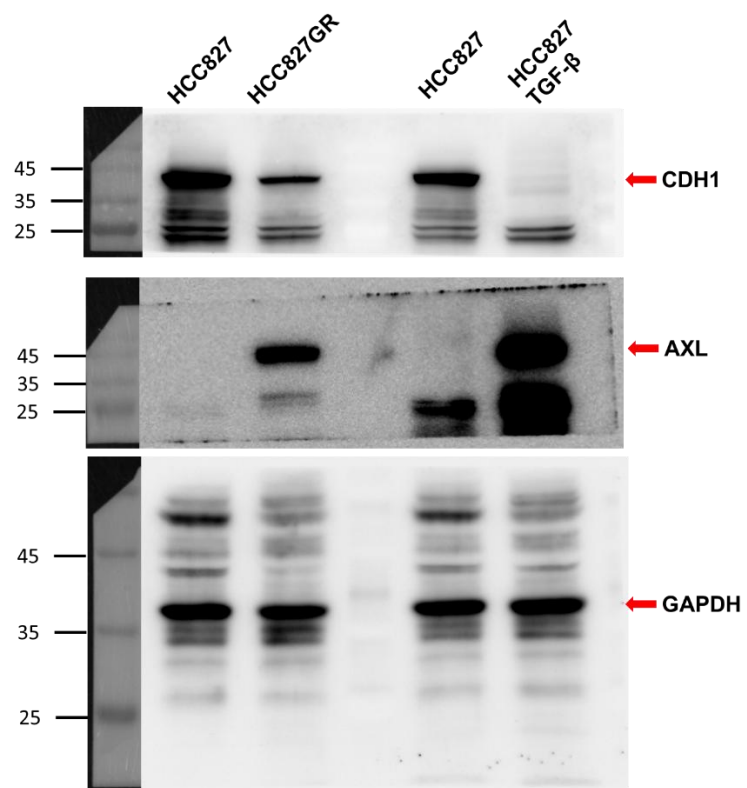
Figure 4F



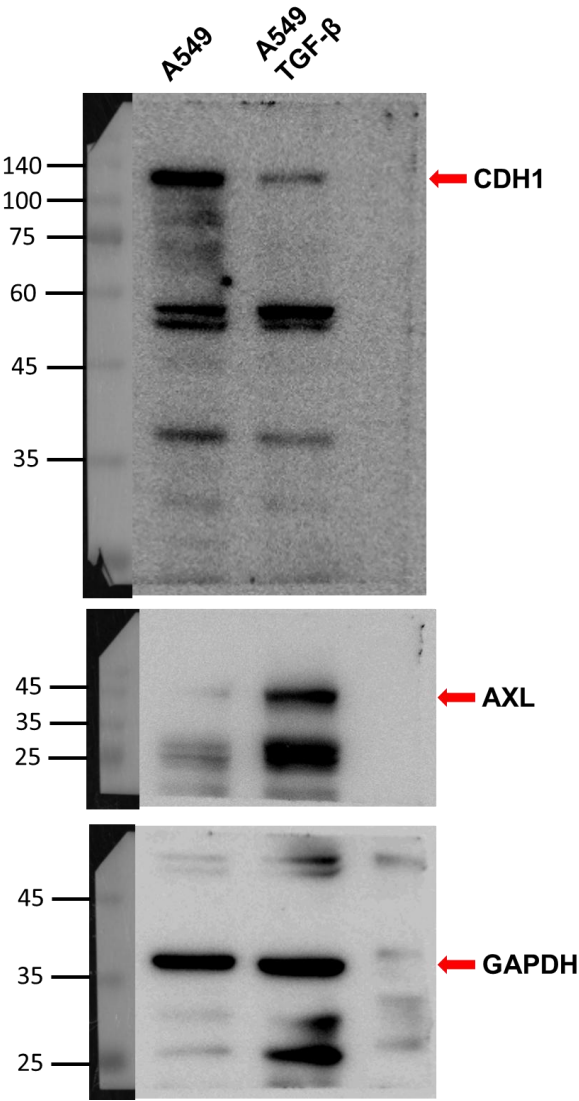
Original Immunoblots
Figure 4G



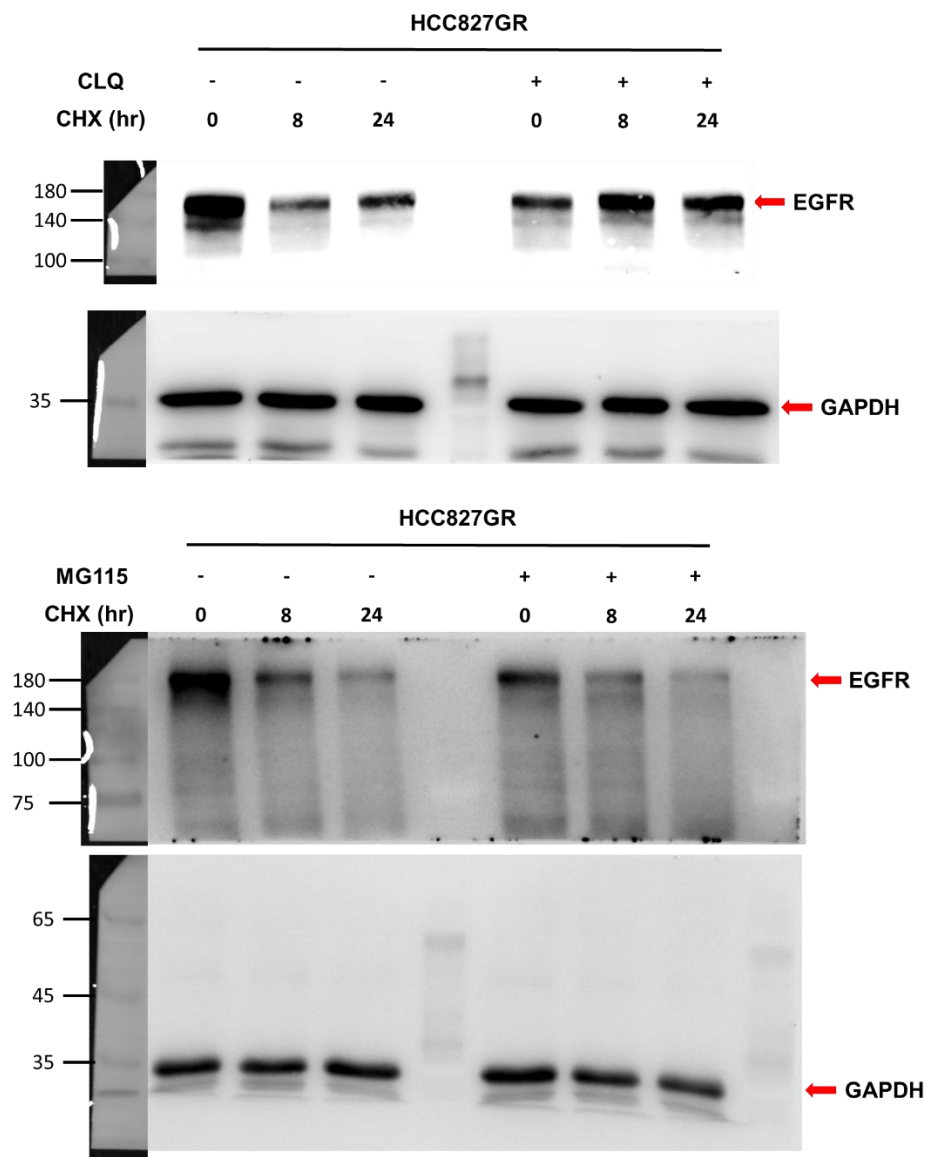
Original Immunoblots
Figures 5E and 6B



Original Immunoblots
Figure 6F



Original Immunoblots
Supplementary Figure 4B



Original Immunoblots
Supplementary Figure 5B

