Supplemental Table 1. Synergistic index of combination treatment with cixutumumab and C225 or rapamycin in MTS assay. LN686 and SqCCY1 cells grown in PCP were treated with indicated concentration of cixutumumab, C225, and rapamycin or their combinations for 3 days. Abbreviation: MGI, mean growth inhibition rate = growth rate of treated group/growth rate of untreated group. *Calculated by dividing the expected growth inhibition rate by the observed growth inhibition rate. An index more than 1 indicates synergistic effect and <1 indicates less than additive effect. † *p* value (two-sided) was calculated by *t* test compared with no treatment. ‡Growth inhibition rate of treatment A x growth inhibition rate of treatment. §Growth inhibition rate of combined treatment on treatments A and B.

Supplemental Table 2. Synergistic index of combination treatment with cixutumumab and C225 in soft agar assay. SqCC/Y1, LN686, UMSCC2, FADU, H226B, H226Br. H460, and H596 cell grown in soft agar were treated with indicated concentration of cixutumumab, C225, or their combination. Abbreviation: MGI, mean growth inhibition rate = growth rate of treated group/growth rate of untreated group. *Calculated by dividing the expected growth inhibition rate by the observed growth inhibition rate. An index more than 1 indicates synergistic effect and <1 indicates less than additive effect. † *p* value (two-sided) was calculated by *t* test compared with no treatment. ‡Growth inhibition rate of treatment A x growth inhibition rate of treatment. §Growth inhibition rate of combined treatment on treatments A and B.

Supplemental Figure Legends

Supplemental Figure 1. Specific inhibition of IGF-I-mediated IGF-1R phosphorylation by cixutumumab. Serum-starved LN686 cells in PCP were pretreated with cixutumumab (25 μg/ml) for 6 h and then stimulated with IGF-I (100

ng/ml) or insulin (100 nM) for 30 min. Cell lysates were analyzed by using Western blot analysis.

Supplemental Figure 2. Cixutumumab-resistant (red-colored) and –sensitive (black-colored) 4 HNSCC and 6 NSCLC cells lines cultured in PCP were analyzed for pIGF-1R, IGF-1R, pEGFR, EGFR, and Actin expressions by using Western blot analysis. Densitometric analysis was performed to quantify band intensity. pIGF-1R (A), IGF-1R (B), pEGFR (C), and EGFR (D) expression levels normalized by Actin expression level are shown.

Supplemental Figure 3. Rapamycin inhibits mTOR and proliferative activities in cixutumumab-resistant HNSCC cells. A, LN686 and OSC19 cells grown in PCP was treated with rapamycin indicated dose for 6h and then stimulated with 10% FBS for 30 min. pmTOR (Y2248) and mTOR were analyzed using Western blot. B, LN686 and SqCC/Y1 cells under PCP were treated with indicated rapamycin concentrations for 3 days and then measured cell viability by using MTS reagents Student's t test, average ± SD; n=4 $p<0.05$ and $p<0.001$.

Supplemental Figure 4. C225 blocks the axis of EGFR signaling. A, LN686 cells grown in PCPs were treated with indicated doses of C225 for 6h and then stimulated with 10% FBS for 30 min. pEGFR (Y1068) and EGFR were analyzed using Western blot. B, LN686 and SqCCC/Y1 cells grown in PCP in the presence of indicated concentrations of C225 for 3 days were subjected to MTS assay. Student's t test, average ± SD; n=6 $p<0.05$ and $p<0.001$.

Supplemental Figure 5. The combined treatment with cixutumumab and C225 significantly decreased cixutumumab induced the activities of EGFR, Akt, and mTOR and activated caspase 3. LN686 and FADU HNSCC cell lines grown in PCPs were treated with cixutumumab (25 μg/ml), C225 (25 μg/ml), or their combination for 3 days and then stimulated with IGF-1 (100 ng/ml) for 30 min. Cell lysates were prepared and subject to immunoblotting analysis.

Supplemental figure 6. Potentiation of proapoptotic effect of cixutumumab by combined treatment with LY294002 or erlotinib. LN686 cells were treated with cixutumumab (25 μg/ml) alone or in combination with LY294002 (10 μM) or erlotinib (100 nM) for 3 days. Cell lysates were prepared and analyzed by Western blot.

Supplementary Table 1.

Synergistic indices of combination treatment with cixutumumab and rapamycin

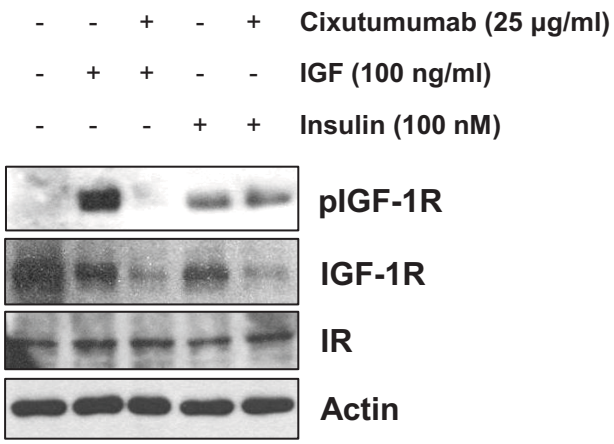
Additive and Synergistic effect												
Cell lines	Treatment A				Treatment B				Combination treatment			Index*
	Drug	Conc.	MGI	*p*-value†	Drug	Conc.	MGI	*p*-value†	Expected‡	Observed§	*p*-value†	
LN686	Cixutumumab	25 μg/ml	0.87	0.040	Rapamycin	1 μM	0.7	0.000	0.61	0.29	0.0001	2.10
SqCC/Y1			0.88	0.072			0.79	0.008	0.70	0.4	0.0001	1.74
LN686			0.84	0.018	C225	25 μg/ml	0.71	0.001	0.60	0.46	0.0023	1.30
SqCC/Y1			0.89	0.043			0.77	0.006	0.69	0.4	0.0000	1.71

Supplementary Table 2.

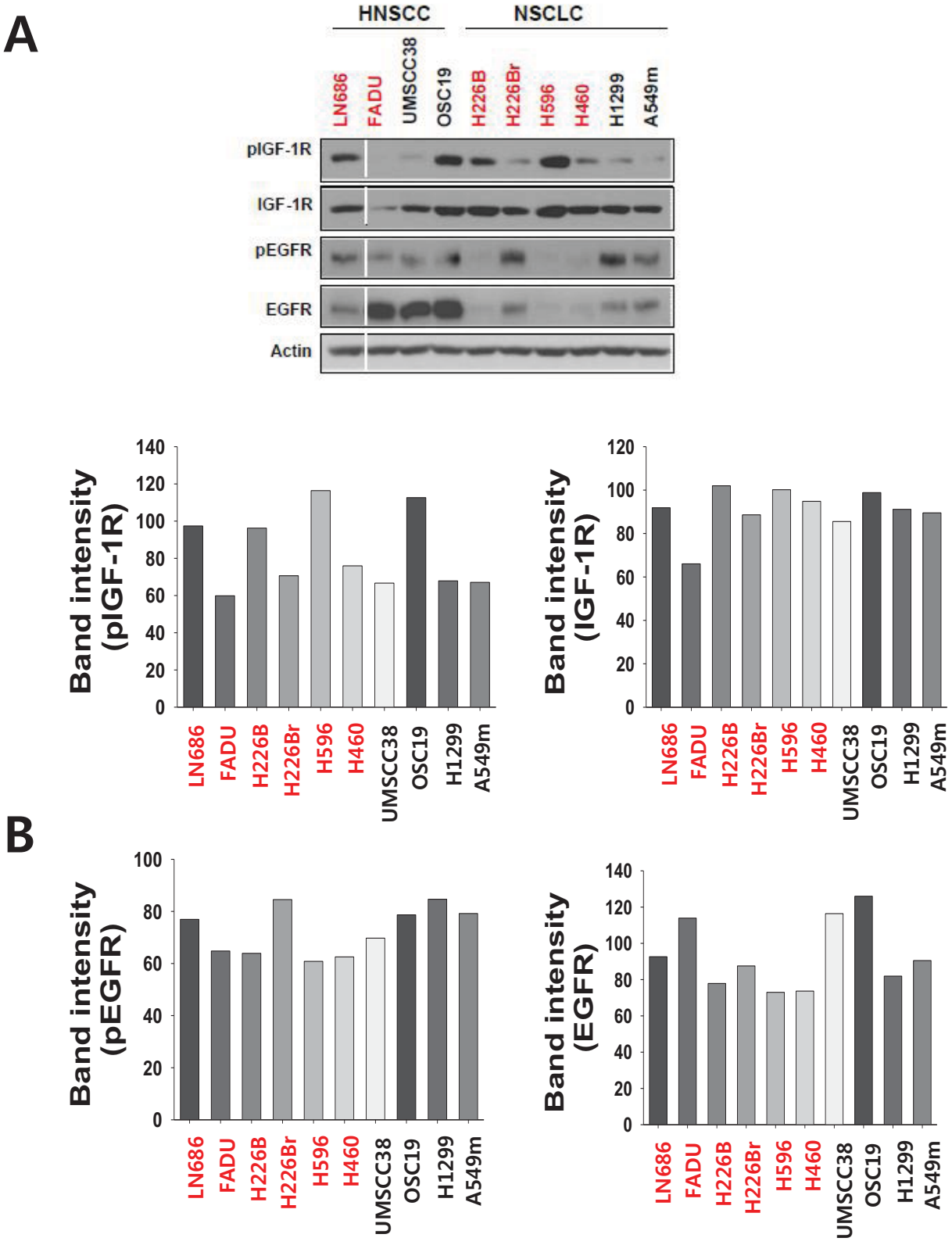
Synergistic indices of combination treatment with cixutumumab and C225

Additive and Synergistic effect												
Cell lines	Treatment A				Treatment B				Combination treatment			Index*
	Drug	Conc.	MGI	*p*-value†	Drug	Conc.	MGI	*p*-value†	Expected‡	Observed§	*p*-value†	
SqCC/Y1	Cixutu mumab	25 μg/ml	0.85	0.00282	C225	25 μg/ml	0.82	0.00052	0.70	0.45	8.08E-09	1.55
LN686			0.63	5.7E-06			0.71	7.9E-06	0.45	0.37	1.87E-08	1.21
UMSCC2			0.79	2.3E-05			0.74	2.9E-05	0.58	0.52	2.84E-10	1.12
FADU			0.54	0.00019			0.42	5E-05	0.23	0.21	1.5E-06	1.08
H226B			0.76	0.006			0.7	0.001	0.53	0.4	5.57E-08	1.33
H226Br			0.59	0.00014			0.68	0.00086	0.40	0.34	1.77E-07	1.18
H460			0.53	8.4E-08			0.64	3.2E-05	0.34	0.32	9.1E-08	1.06
H596			0.61	6.3E-08			0.59	1.2E-05	0.36	0.31	1.29E-07	1.16

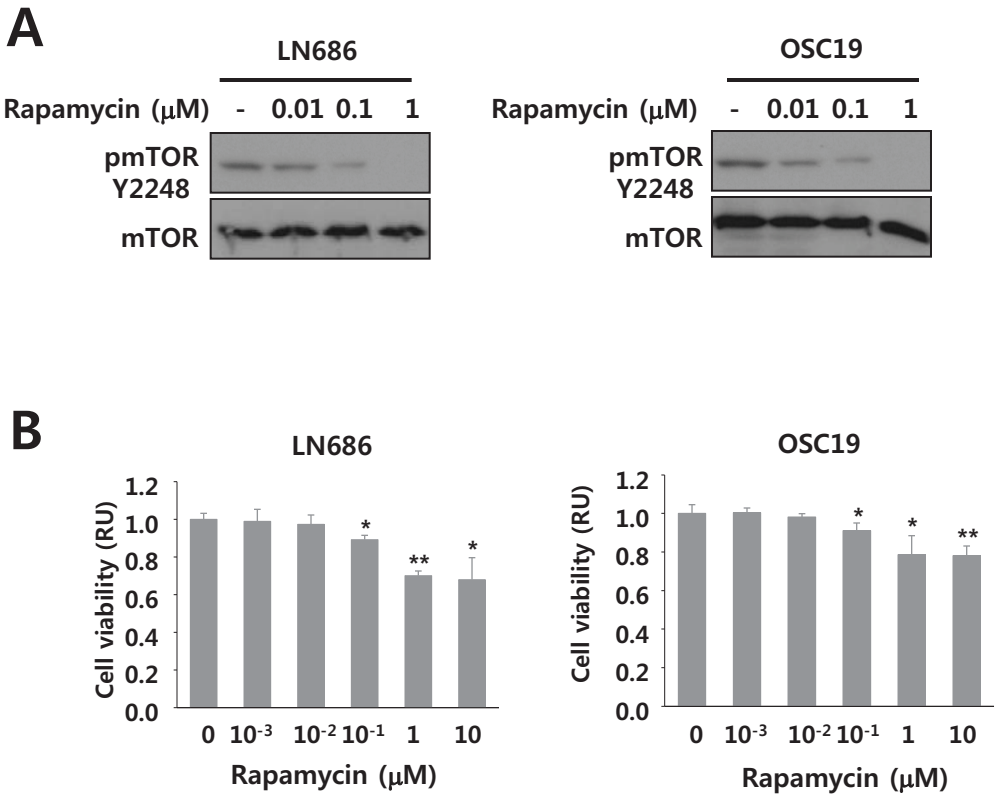
Supplementary Figure 1



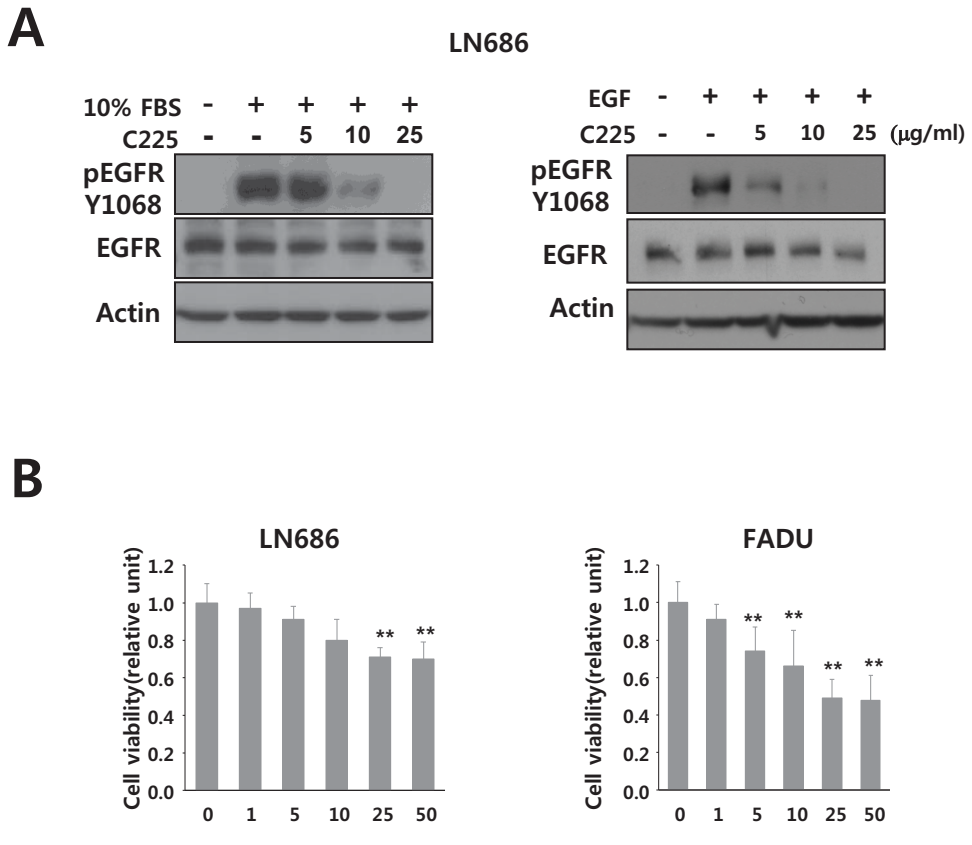
Supplementary Figure 2



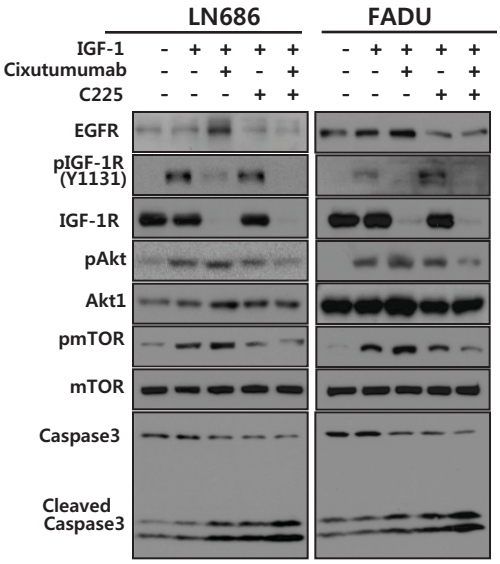
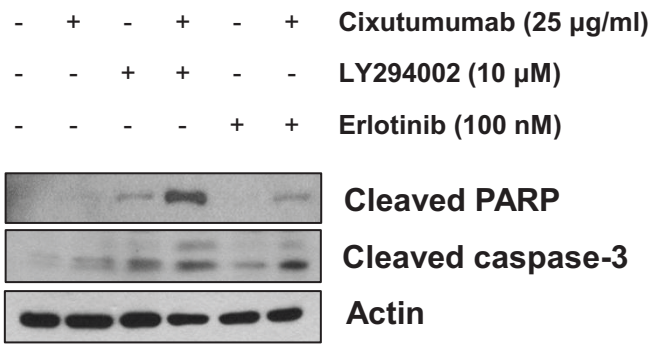
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6