

**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  $\pm$  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  $\pm$  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  $\mu\text{g/ml}$ ), C225 (25  $\mu\text{g/ml}$ ), 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  $\mu\text{g/ml}$ ) alone or in combination with LY294002 (10  $\mu\text{M}$ ) 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 <sup>c</sup>
	Drug	Conc.	MGI	p-value <sup>†</sup>	Drug	Conc.	MGI	p-value <sup>†</sup>	Expected <sup>‡</sup>	Observed <sup>§</sup>	p-value <sup>†</sup>	
LN686	Cixutu mumab	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

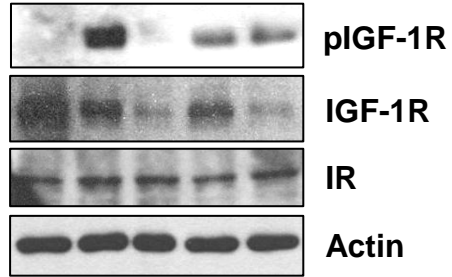
## Supplementary Table 2.

Synergistic indices of combination treatment with cixutumumab and C225

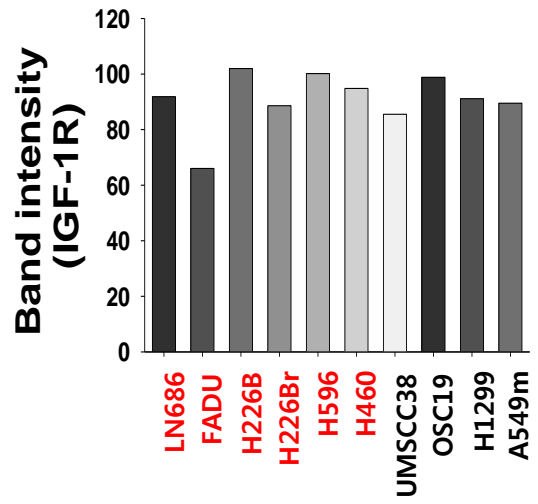
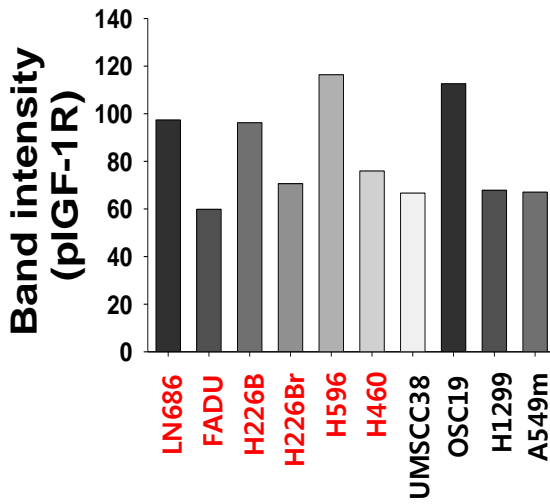
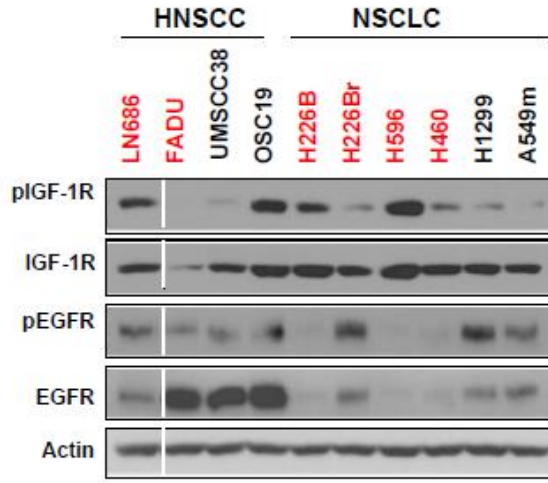
Additive and Synergistic effect												
Cell lines	Treatment A				Treatment B				Combination treatment			Index*
	Drug	Conc.	MGI	p-value†	Drug	Conc.	MGI	p-value†	Expected‡	Observed§	p-value†	
SqCC/Y1	Cixutu mumab	25 µg/ml	0.85	0.00282	C225	25 µg/ml	0.82	0.00052	0.70	0.45	8.08E-09	1.55
LN686			0.63	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

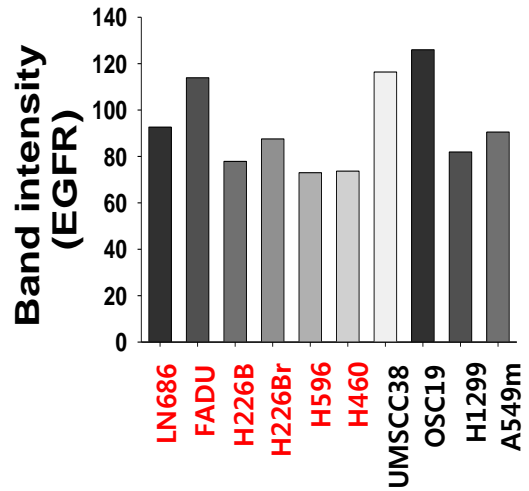
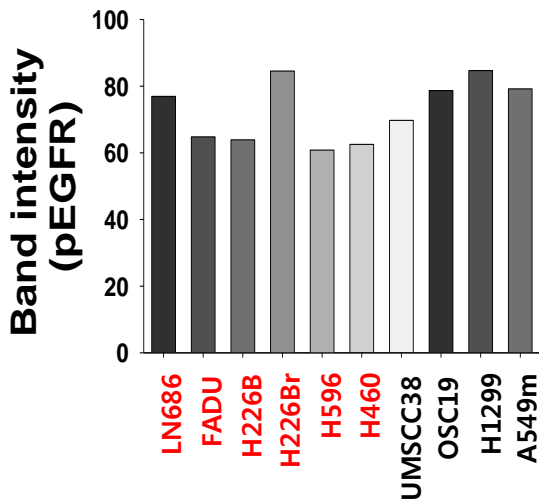
-	-	+	-	+	Cixutumumab (25 $\mu$ g/ml)
-	+	+	-	-	IGF (100 ng/ml)
-	-	-	+	+	Insulin (100 nM)



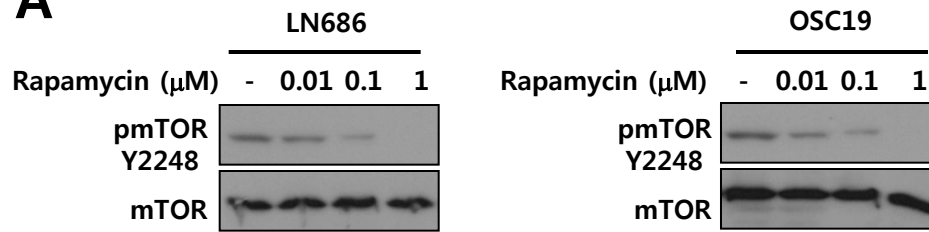
**A**



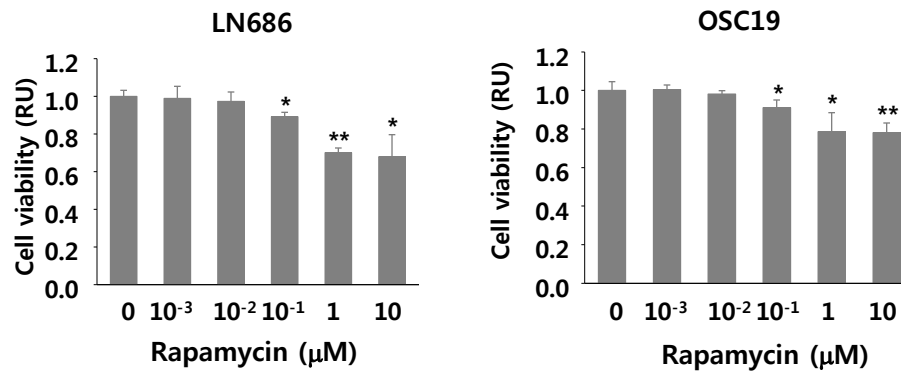
**B**



**A**

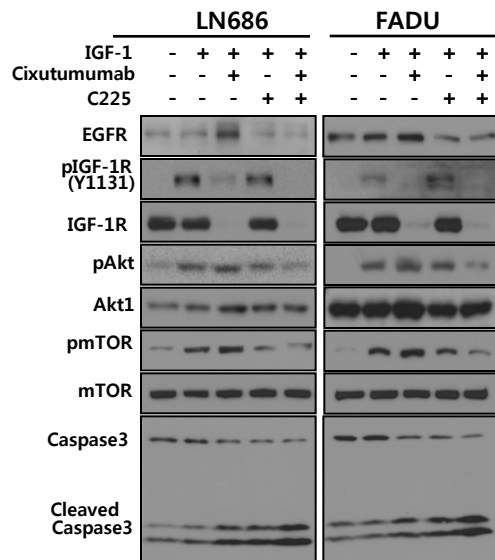


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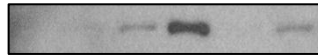




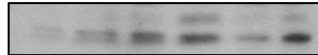


**Supplementary  
Figure 6**

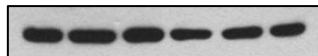
-	+	-	+	-	+	<b>Cixutumumab (25 µg/ml)</b>
-	-	+	+	-	-	<b>LY294002 (10 µM)</b>
-	-	-	-	+	+	<b>Erlotinib (100 nM)</b>



**Cleaved PARP**



**Cleaved caspase-3**



**Actin**